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AN ELECTROPHYSIOLOGICAL STUDY OF PERIPHERAL AND
CENTRAL CARDIOMOTOR ACTIVITY OF THE VAGUS NERVE

BY

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A THESIS

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The undersigned certify that they have read,
and recommend to the Faculty of Graduate Studies for
acceptance a thesis entitled AN ELECTROPHYSIOLOGICAL
STUDY OF PERIPHERAL AND CENTRAL CARDIOMOTOR ACTIVITY
OF THE VAGUS NERVE submitted by Franco Romano Calaresu
in partial fulfilment of the requirements for the
degree of Doctor of Philosophy.

ABSTRACT

Five groups of experiments were carried out in cats to obtain data on central and peripheral patterns of discharge in the efferent limb of the reflex arc producing cardiac slowing and to determine the site of origin of vagal cardioinhibitory fibers.

Recordings were obtained from 285 "single" efferent fibers in the cervical vagus. None of these fibers exhibited a cardiac rhythm. 7 fibers increased their rate of discharge in time with phenyldiguanide (PDG) induced bradycardia.

The "collision technique" was used in 30 experiments.

Changes in the compound action potential of the cervical vagus before, during and after PDG induced bradycardia were not observed.

252 stainless steel microelectrode penetrations aimed at the dorsal nucleus of the vagus (DNV) were made and iron was deposited at the site of recording. 50 successful penetrations in the DNV were obtained. 6 units showed increased activity after PDG and in time with bradycardia and 1 showed increased activity in time with bradycardia following carotid sinus stimulation. None of the units exhibited a cardiac rhythm. The function of the fibers in the cervical vagus and of the units in the DNV which increased their rate of discharge in time with bradycardia was not established but their behavior is consistent with that expected in cardioinhibitory fibers and units.

Lesions of the DNV were produced in 5 cats. Axonal degeneration was demonstrated in the intramedullary rootlets of the vagus and in the cervical vagus of all animals ;degeneration

could be demonstrated in cardiac branches of only 2 of these animals. It is concluded that anatomical connections exist between DNV and cardiac motor fibers.

Bipolar stimulation of medullary structures followed by histological localization was carried out in 38 cats. Stimulation of 22 sites within the nucleus of the tractus solitarius (NTS) or the tractus solitarius (TS) produced bradycardia and a compound action potential in the ipsilateral cervical vagus ; they both persisted unchanged following section of the ipsilateral vagus. Two distinct locations within the NTS and TS were identified : their electrical stimulation produced bradycardia and two distinct types of compound action potential in the ipsilateral cervical vagus. Stimulation of the more rostral of the two locations produced a compound action potential with a latency of 1.2 msec and the more caudal produced a compound action potential with a latency of 2 msec . It is concluded that first order afferent fibers in the NTS or the TS were being stimulated.

Stimulation of 10 sites within the DNV failed to produce any changes in heart rate, arterial pressure or respiration but, on three occasions, produced a small compound action potential in the ipsilateral cervical vagus. It is suggested that stimulation of the DNV failed to produce bradycardia because an adequate number of cardioinhibitory units was not excited.

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TABLE OF CONTENTS

	Page
ABSTRACT	I
ACKNOWLEDGEMENTS	III
LIST OF TABLES	VII
LIST OF FIGURES	VIII
INTRODUCTION	2

HISTORICAL REVIEW

A. The vagus nerve and heart rate	7
B. Fiber composition of the vagus nerve	13
C. Recordings from cardiac vagal efferents	18
D. Histology of the dorsal nucleus of the vagus	24
E. Neurophysiology of the dorsal nucleus of the vagus	31
F. Conclusions and statement of objects of research	38

METHODS

A. Recordings from vagal efferent fibers	40
(1) "Single" fiber technique	41
(2) "Collision technique"	42
B. Recordings from the dorsal nucleus of the vagus	42
(1) Mapping of the nucleus	42
(2) Physiological experiments	43
(3) Histological localization of the sites of recording	45

C.	Lesions of the dorsal nucleus of the vagus	47
	(1) Surgical procedure	47
	(2) Histological techniques	48
D.	Stimulation of the medulla	49
	(1) Physiological experiments	49
	(2) Histological localization of the sites of stimulation	50
	(3) Analysis of compound action potentials	50

RESULTS

A.	Recordings from vagal efferent fibers	52
	(1) "Single" fibers in the cervical vagus	52
	(2) "Collision technique"	53
B.	Recordings from the dorsal nucleus of the vagus	53
C.	Lesions of the dorsal nucleus of the vagus	56
D.	Stimulation of the medulla	58
	(1) Systematic exploration	58
	(2) Stimulation of selected points	58

DISCUSSION AND CONCLUSIONS

A.	Recordings from vagal efferent fibers	62
B.	Recordings from the dorsal nucleus of the vagus	66
C.	Lesions of the dorsal nucleus of the vagus	69
D.	Stimulation of the medulla	72
E.	General discussion and conclusions	77

SUMMARY	86
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REFERENCES	90
------------	----

LIST OF TABLES

Table No.		Following Page
I	Conduction velocities of vagal cardiomotor fibers.	10
II	Efferent activity in cardiac branches of the vagus.	22
III	Histological studies on the dorsal nucleus of the vagus.	30
IV	Physiological studies on the dorsal nucleus of the vagus.	36
V	Physiological characteristics of some efferent fibers in the cervical vagus.	53
VI	Physiological characteristics of some units in the dorsal nucleus of the vagus.	54

LIST OF FIGURES

Figure No.		Following page
1.	Diagram of medulla and vagus nerve in the cat.	13
2.	Autonomic innervation of the heart in the cat.	22
3.	Dorsal nucleus in a longitudinal section of the medulla.	24
4.	Map of the dorsal nucleus.	24
5.	Facsimile of a paper by the Weber brothers.	32
6.	Activity from an efferent fiber in the cervical vagus.	42
7.	Activity from medullary units.	45
8.	Experimental arrangement used for stimulation of the medulla.	50
9.	Activity from a laryngeal motor fiber and from an aberrant baroreceptor fiber.	52
10.	An efferent fiber whose activity increased in time with bradycardia	53
11.	Frequency of discharge of a fiber and heart rate versus time.	53
12.	An efferent fiber whose activity decreased in time with bradycardia.	53
13.	Representative "collision technique" experiment.	53

Figure No.		Following page
14.	Location and type of electrical activity of 50 units in the dorsal nucleus.	54
15.	A dorsal nucleus unit whose activity increased in time with bradycardia.	54
16.	Frequency of discharge of a dorsal nucleus unit and heart rate versus time.	54
17.	Iron deposit within the dorsal nucleus.	54
18.	Outlines of lesions of the dorsal nucleus.	56
19.	Size and location of a lesion of the dorsal nucleus.	56
20.	Axonal degeneration in intramedullary rootlets after lesion of the dorsal nucleus.	57
21.	Axonal degeneration in the cervical vagus after lesion of the dorsal nucleus.	57
22.	Axonal degeneration in the cervical vagus after infranodose vagotomy.	57
23.	Size and location of a lesion of the nucleus solitarius.	57
24.	Axonal degeneration in a cardiac branch of the vagus.	58
25.	Lesions of the dorsal nucleus and amounts of axonal degeneration in the vagus nerve.	58

Figure No.		Following page
26.	Representative compound action potentials in the vagus and physiological responses during stimulation of points on the surface of the medulla.	58
27.	Representative compound action potentials in the vagus and physiological responses during stimulation of points 1 mm below the surface of the medulla.	58
28.	Physiological responses during stimulation of points on the surface of the medulla.	58
29.	Physiological responses during stimulation of points 1 mm below the surface of the medulla.	58
30.	Location of iron deposits in the nucleus and the tractus solitarius.	59
31.	Location of iron deposits in the dorsal nucleus.	59
32.	Two iron deposits within the dorsal nucleus.	59
33.	Bradycardia before and after ipsilateral vagotomy.	60
34.	Outlines of two distinct areas within the nucleus and the tractus solitarius.	60
35.	Compound action potentials in the vagus during stimulation of two distinct areas in the nucleus and the tractus solitarius.	61
36.	A compound action potential in the vagus during stimulation of the dorsal nucleus.	61

Figure No.		Following page
37.	Hypothetical course of aberrant baroreceptor fibers.	63
38.	Compound action potentials in the vagus after ipsilateral vagotomy.	74
39.	Stimulation of the dorsal nucleus at five different frequencies.	83
40.	Stimulation of the dorsal nucleus at different voltages.	83

""VAGUS UND HERZ, - SEIT DEN TAGEN ED. WEBER'S
BIS AUF DIE UNSRIGEN EIN THEMA VON
UNERSCHÖPFLICHER ANZIEHUNGSKRAFT FÜR DIE
UNTERSUCHUNG!""

R. HEIDENHAIN

""THE IDEAL APPROACH TO THE PROBLEM OF NEURAL
CODING IS TO OBTAIN THE OUTPUT FOR ALL
RANGES AND KINDS OF INPUT, AND TO DO THIS
FOR SINGLE JUNCTIONS, SINGLE NEURONS ACTIVATED
THROUGH ALL COMBINATIONS OF JUNCTIONS AND
NEURON POOLS RECEIVING IMPULSES FROM VARIOUS
GROUPS OF AFFERENT FIBERS.""

R. W. GERARD

INTRODUCTION

The available knowledge about the nervous integration of cardiovascular reflexes is scarce. Most of the data in the literature are concerned with the recording of cardiovascular responses following stimulation or section of peripheral nerves or stimulation or ablation of areas of the central nervous system. These data provide only a substitute for the description of flow of information in afferent and efferent fibers and in synaptic locations.

Some reasons may be given to explain why the electrophysiological data are so few. Isolation and identification of many of the fibers involved in cardiovascular reflexes is very difficult because of their small size. These fibers do not follow specific anatomical routes but are usually intermingled with fibers subserving other functions. The cell stations of cardiovascular reflex arcs are widely scattered and not well known. The locations from which some of the electrophysiological data have been obtained have been determined by stereotaxic coordinates and may therefore be questioned. Finally, it is difficult to predict a priori the meaning of patterns of discharge which are not related to known and easily observable body phenomena.

A large flow of impulses from cardiac and pulmonary afferents has been recorded from the vagus nerve and these impulses are generally believed to influence in an unknown manner the discharge of efferent neurones in the

medulla oblongata (Schaefer, 1960).

The medulla, as a result of studies of the effect on cardiovascular events of serial transection and ablation and stimulation of medullary structures, has emerged as a structure containing a system of neurones, intercalated between the afferent and the efferent fibers, which coordinate cardiovascular reflexes. Most of the evidence for such a function has been provided by the experiments of Alexander (1946), Bach (1952), Hoff, Breckenridge and Spencer (1957) and Glasser (1962). A general limitation of these studies is that their interpretation is made difficult because ablation and stimulation are rather coarse techniques and comparisons between data obtained by different investigators are not always possible. Furthermore, results from ablation experiments do not indicate the precise physiological function of a certain structure in an intact circuit, but rather allow inferences on what the function might be by producing either release of function or depression of function of related components of the circuit. In an attempt to throw some light on this subject three separate groups of workers have recently attempted to record from single units in the medulla oblongata.

Smith & Pearce (1961) have recorded single unit activity with a cardiac rhythm from nine units in the medulla near the obex in the region of the nucleus solitarius; these discharges did not resemble any of the known patterns of discharge of the primary afferent vagal fibers. These authors

concluded that they were recording from second order afferent neurones and that "the rhythmic pattern of discharge of cardiovascular afferents is drastically modified at their first synapse".

Hellner & v. Baumgarten (1961) have recorded activity with a cardiac rhythm of three types comparable to activity found in peripheral vagal afferents. The discharges, from an undetermined number of units, were most commonly found in a well defined area dorsal or dorsolateral to the tractus solitarius "one to three millimeters rostral to the obex and one millimeter lateral to the edge of the fourth ventricle". The site of recording was marked by an electrolytic lesion. They concluded that the recorded activity, from what they believed to be second order afferent neurones, maintained the timing characteristics of vagal afferents.

Salmoiraghi (1962) has recorded activity with a cardiac rhythm from six units "in a region (1-2 mm cranial to the obex and 1-3 mm lateral to the midline, at depths from 0.5 to 1.2 mm), probably corresponding to that of the nuclei sensibiles of the ninth and tenth cranial nerves and/or the tractus solitarius"; he agreed with Hellner & v. Baumgarten about the function and nature of these units. Furthermore Salmoiraghi has also recorded from fifty-three units widely scattered in the medulla and the pons which varied their frequency of discharge with spontaneous changes in arterial

pressure and suggested that these neurones, as they did not exhibit any recognizable rhythm, were integrating neurones in the pathway of the cardiovascular reflex arc.

From the three publications just summarized it would be premature to outline a scheme of integration of cardiovascular reflexes because of the very limited amount of available evidence, the diversity of discharge patterns described by the different authors and the unrefined methods of localization used by Salmoiraghi and Smith & Pearce.

When the present work was begun little information was available on either central or peripheral vagal efferent discharge patterns to the heart, and, as will appear in the historical review, there is still some controversy over the site of origin of the cardiomotor neurones. The recording in central and peripheral cardiac efferents of spontaneous discharges and of discharges following procedures which induce slowing of the heart would eliminate many of the problems of interpretation discussed in relation to experiments of ablation and electrical stimulation. Furthermore, Bronk, Ferguson & Solandt (1934) have made a careful analysis of the effects of carotid sinus stimulation on the activity of cardiac sympathetic nerves, but no information on the impulse activity in the cardiac vagal efferents following carotid sinus stimulation is available. A final and cogent justification for the present undertaking is that the study of neural coding, applied to the cardiovascular system, may provide information of

general importance in the understanding of nervous integration. Even though the neural apparatus controlling cardiovascular reflexes appears to be a complex system, it still remains a good choice for the study of neural coding for at least two reasons. Firstly, the physiology of spinal reflexes has been satisfactorily elucidated and the study of the first supraspinal reflex "station", namely the medulla, seems to be the obvious next field of study. Secondly, as neural regulatory apparatuses controlling the circulatory system are present in a wide range of animal phyla, it seems reasonable to assume that they may prove easier to attack experimentally than higher brain structures and functions, some of which are present only in a limited number of animal phyla.

In summary, in spite of the intricacies of the neural regulation of cardiovascular reflexes, an orderly functional organization must exist, because specific responses can usually be observed following a specific stimulus. Recognizing the limitations listed earlier, an investigation was started in the attempt to obtain electrophysiological data from the efferent limb of the vegetative reflex arc which produces slowing of the heart.

HISTORICAL REVIEW

A. The vagus nerve and heart rate.

The concept of the nervous control of heart rate is a relatively recent one. William Harvey (1628) believed in the complete autonomy of the heart and in the "Exercitatio Anatomica" had this to say:

"... quod cor nempe primum subsistens sit, et habeat in se sanguinem, vitam, sensum, motum..."

The Weber brothers (1845) were the first to describe slowing of the heart following electrical stimulation of the vagus in frogs and rabbits. Donders (1868) studied the effect on the heart rate of repetitive and single electrical stimuli to the vagus nerves of rabbits and dogs and showed that both types of stimuli prolonged the interval between contractions. Heidenhain (1882), by stimulating the vagus of the frog with induction shocks or by placing sodium chloride crystals on the nerve obtained cardioacceleratory as well as cardioinhibitory responses. Hunt (1898-1899) studied the effect of cutting the sympathetic accelerator nerves on the cardioinhibitory activity of vagal stimulation in dogs, rabbits, and cats and concluded that accelerator nerves were in continuous tonic activity. Heinbecker (1931) showed that stimulation of the peripheral cut end of the vagus nerve produced slowing of the heart in the turtle and in the frog only when the C component was present

in the recorded compound action potential. Brown & Eccles (1934a) by applying single induction shocks to the peripheral end of the transected vagi in decerebrate cats produced slowing of the heart which persisted for many cardiac cycles. By using stimuli to the vagus just above and below the threshold for the inhibition of cardiac activity and recording the evoked compound action potential they measured an average conduction velocity of 30 meters per second in what they considered to be the B₂ fibers producing bradycardia. Brown & Eccles (1934b) showed the absence of a refractory period in the heart of cats following stimulation of either vagus, and concluded that the right and left vagi were distributed to different pacemaker cells. They also showed that the inhibitory effect of stimulation of the right vagus was greater than that of the left vagus and that simultaneous stimulation of the two produced an inhibition of cardiac activity which was the sum of the two separate inhibitions. Rosenblueth & Simeone (1934) studied the interrelation of stimulation of vagus and sympathetic on heart rate and concluded that the two effects occurred independently. Heinbecker & Bishop (1935) showed that in cats and rabbits the negative chronotropic effect on the heart was initiated on excitation of B₂ fibers and almost completed by excitation of B₃ fibers. A slight increase of the chronotropic effect was frequently obtained on stimulation of the C fibers. They also maintained that the fibers involved in producing bradycardia were in the

range 1.5 to 4.5 micra in diameter and disagreed with Brown & Eccles' findings regarding conduction velocity.

They stated:

"... The fastest of the autonomic motor fibers of the cat vagus conduct at not over 15 meters per second and those usually affecting the heart at not over half of that. ..."

Middleton, Middleton & Grundfest (1950) studied the effect of vagus stimulation on the "isolated in situ" heart of cats kept at a temperature between 26° and 29°C., and concluded that the cardioinhibitory effect on the heart was mediated mainly by fibers of the B group with a maximal conduction velocity of 14.3 meters per second and that stimulation of C fibers did not increase the cardioinhibitory effect. Stimulation of the vagus nerve following supranodose vagotomy, after enough time had been allowed for motor fibers to degenerate, failed to affect cardiac activity even though the compound action potential of the degenerated nerve was similar to that of the normal nerve. To explain these results the authors suggested that the vagal motor fibers to the heart were a very small proportion of the total number of fibers in the vagus. Recent histological work by Agostoni, Chinnok, Daly & Murray (1957), indicating that myelinated fibers to the heart are very few, seems to support their explanation. Daly & Evans (1953) showed that in cats, after chronic supranodose or infranodose vagotomy, stimulation of the peripheral end of the vagus caused no effect on the heart and concluded that there were

no motor fibers to the heart with their cell bodies in the nodose ganglion. They also stated:

"... Our histological findings indicate that motor functions of the vagus to the heart and bronchial musculature are conveyed essentially by myelinated fibers of the 2-4 micra diameter group and by non-myelinated fibers. ..."

A similar study was made in the rabbit by Evans & Murray (1954) and their conclusions were similar to those of Daly & Evans in the cat. Agostoni, Chinnok, Daly & Murray (1957) showed that in cats electrical stimulation of the peripheral cut end of the vagus produced arterial hypotension, bradycardia and contraction of the stomach and duodenum; the same effects could not be obtained in cats after a suitable period of time following either supranodose or infranodose vagotomy. Koepchen, Wagner & Lux (1961), using a modification of the Helmholtz technique for measuring conduction velocity in nerves to skeletal muscles, showed that the conduction rate for cardiomotor fibers in the cat was between 3.8 and 6.3 meters per second. They also showed that some cardiac slowing could be obtained following stimulation of the vagus nerve cooled to 1°C. : this suggests that some C fibers are involved in cardiac inhibition.

The available data on conduction velocity of vagal cardiomotor fibers are presented in table I.

In summary, it seems well established that stimulation of the peripheral end of the cut vagus nerve of the cat produces bradycardia. From the analysis, by different authors, of the compound action potential of

TABLE I

CONDUCTION VELOCITIES OF VAGAL CARDIOMOTOR FIBERS

Authors and year	Species	Technique	Conduction velocity in mps
Brown & Eccles (1934a)	Cat	compound action potential	30 (average)
Heinbecker & Bishop (1935)	cat and rabbit	compound action potential	< 7.5
Middleton <u>et al.</u> (1950)	cat	compound action potential	23* (maximum)
Koepchen <u>et al.</u> (1961)	cat	Helmholtz technique	4.6 (average) possibly C fibers

* Corrected for temperature

the vagus nerve it seems that, to obtain bradycardia, at least the component referred to as "B" must appear. Also, from the work of Heinbecker & Bishop and Koepchen et al., it appears that C fibers may be involved in cardioinhibition. The values for conduction velocity of cardiomotor fibers obtained by Brown & Eccles of approximately 30 meters per second, and those of Middleton et al. corrected for temperature (assuming a Q_{10} of approximately 1.7 (Gasser & Erlanger, 1927)) are of comparable magnitude. Such a conduction velocity would indicate that the fibers involved belong to the A group, according to the standard classification proposed by Gasser (1941). As Brown & Eccles and Middleton et al. state categorically that the vagal motor fibers to the heart belong to the B group, it is impossible to reconcile their statement with the commonly accepted classification which puts the upper limit for conduction velocity of B fibers at approximately 15 meters per second. In contrast to these values, the values given by Heinbecker & Bishop and by Koepchen et al. are much smaller. It is very difficult to reconcile this divergence of findings. Heinbecker & Bishop's measurements were obtained from isolated vagi in a chamber, and the temperature in the chamber may have been considerably lower than the body temperature, but no technical flaw can be found in the results of Koepchen et al..

Even though we have no unequivocal data on conduction velocity in cardiomotor fibers it seems reasonable to conclude from the work of Middleton et al., that the

number of cardiomotor fibers is so small that their presence or absence is not easily detected in a compound action potential. Furthermore it appears that the measurement of conduction velocity from compound action potentials is not a very satisfactory method. We must then consider the different values with caution and wait for more accurate experimental determinations. The values obtained by Koepchen et al., obtained with a more reliable technique, should be regarded as the best available measurements of conduction velocity to date.

B. Fiber composition of the vagus nerve.

The vagus is a mixed nerve originating in the medulla oblongata and sending branches to practically all viscera. An outline of its supradiaphragmatic distribution in the cat is shown in Fig. 1.

Chase & Ranson (1914) demonstrated in the vagus nerve of one cat that the number of unmyelinated fibers exceeded that of myelinated fibers. Heinbecker (1930) recorded compound action potentials from isolated sympathetic and vagus nerve trunks of the turtle and the cat and showed that most of the nerves exhibited A, B₁, B₂ and C elevations and confirmed these results by a cursory histological examination. Jones (1932) examined the pyridine silver and osmic acid stained cross sections of the vagus nerve of one cat and counted approximately 7900 fibers (5400 myelinated) above the nodose ganglion and approximately

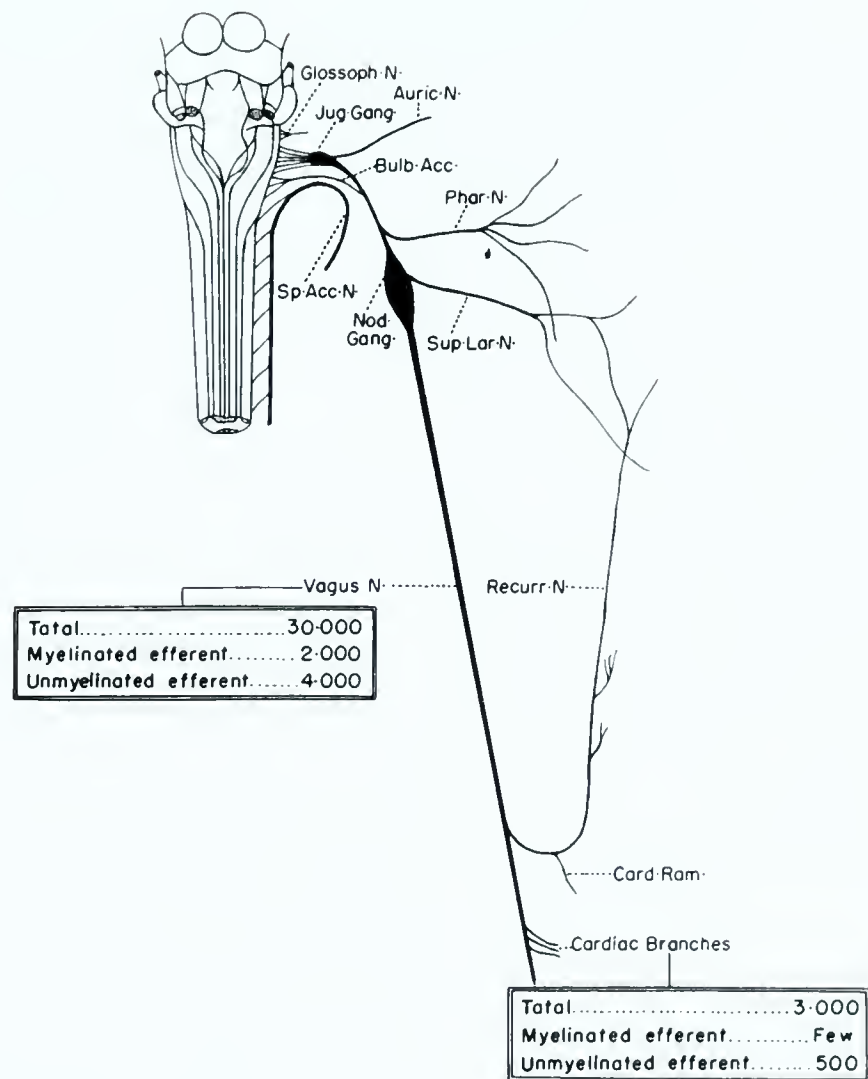


Fig. 1. Schematic diagram of medulla and vagus nerve in the cat (modified from Du Bois & Foley, 1936). Number and type of fibers present in the cervical vagus and its cardiac branches are also shown (figures from Du Bois & Foley, 1936 ; Daly & Evans, 1953 ; Agostoni et al., 1957).

11900 (2450 myelinated) below the nodose ganglion and concluded that a large number of unmyelinated fibers originated from cell bodies in the ganglion. Morgan & Goland (1932) from their studies on the composition and function of the vago-sympathetic trunk in the dog concluded that probably there were cells in the nodose ganglion giving rise to post-ganglionic parasympathetic fibers which when stimulated produced hypotension and sometimes bradycardia. Richardson & Hinsey (1933) could not produce bradycardia in cats following chronic supranodose vagotomy and concluded that there were no cardiomotor parasympathetic preganglionic neurons in the nodose ganglion. Ranson, Foley & Alpert (1933) differentiated efferent fibers in the vagus nerve of cats into large and small myelinated and small unmyelinated. Also, in disagreement with Jones' findings, they counted an equal number of unmyelinated fibers above and below the nodose ganglion. Foley (1934) from histological studies of normal vagi in cats concluded that fibers in the trunk below the pharyngeal and the two laryngeal branches were predominantly unmyelinated. Du Bois & Foley (1936) studied the degenerated motor fibers of the vagus in the cat following supranodose vagotomy. The total number of fibers above the nodose ganglion ranged from 26,000 to 33,500 and the number of myelinated and unmyelinated motor and sensory axons in the cervical vagus and in the auricular, pharyngeal and superior and inferior laryngeal nerves was also determined. Foley & Du Bois (1937) established from studies in two normal cats

that the total number of fibers in the cervical vagus was approximately 33,000 and 39,000. After studying nine cats after supranodose vagotomy they concluded that approximately 80% of the fibers were sensory, of which 10 to 20% were myelinated; of the motor fibers 48 to 71% were myelinated. Daly & Evans (1953) showed that in cats section of the vagus below the nodose ganglion produced degeneration of nearly all fibers in the cervical vagus trunk and in its cardiac and bronchial branches. Furthermore, after supranodose vagotomy there was no obvious reduction, from the value in intact cats, in the number of myelinated fibers in the cardiac branches, indicating that cardiac branches contained very few, if any, myelinated efferents. Evans & Murray (1954) studied the fiber composition of the vagus nerve of the rabbit and concluded that the cervical vagus contained approximately 23,000 fibers, of which only about 13% were myelinated and between 20 and 25% were efferents. They also concluded that the cardiac branches of the vagus contained afferent and efferent fibers of both the medullated and unmedullated varieties. Hoffman & Kuntz (1957) studied the vagus nerves of cat and man. They concluded that in the cat $\frac{2}{3}$ to $\frac{3}{4}$ of all vagal fibers were unmyelinated and that the cardiac rami were composed chiefly of myelinated fibers of less than 7 micra in diameter. They also showed that a large number of ganglion cells were present within the vagus trunk. Agostoni et al. (1957) established that each cervical vagus of the cat contained approximately

30,000 fibers of which about 20% were efferent. Approximately 5,000 of the total number of fibers and approximately 2,000 of the efferent fibers were myelinated. In two normal cats studied the total number of fibers in the left cardiac branches was approximately 3,000, of which 500 were myelinated. In two other cats after supranodose vagotomy there was no change in the number of myelinated fibers of the left cardiac branches, but the number of degenerated unmyelinated efferents was approximately 500 (see Fig. 1). With regard to the cardiac branches the authors stated:

"... of the considerable number of the individual cardiac branches examined after supranodose or intracranial vagotomy, sections taken from five branches and stained by the Weigert method showed a few scattered myelin droplets. This suggests that a small number of myelinated fibres had degenerated and were therefore efferent. Because of the variation in the number of myelinated fibres in the cardiac branches, the loss of a small number after supranodose vagotomy may well not be reflected in the few counts made. ..."

It is important to note that these data apply only to the left cardiac branches and there is the possibility that the fiber composition of the right branches might be slightly different, as a more pronounced cardiac slowing can be produced by stimulation of the right vagus (Brown & Eccles, 1934b). Hoffman & Schnitzlein (1961) examined the mid-cervical vagus trunks from human autopsy material in 17 cases using the pyridine silver stain and obtained a mean of approximately 105,000 fibers for the right trunk and approximately 87,000 fibers for the

left trunk; 80% of the fibers were unmyelinated.

To summarize, it seems well established that the total number of fibers in the vagus nerve of the cat is approximately 30,000, of which approximately 5,000 are myelinated and approximately 2,000 of these are efferent. All the recent workers in the field seem to be of the opinion that the nodose ganglion does not contain cell bodies of motor fibers, at least in the cat, and that the percentage of motor fibers in the cervical vagus is approximately 20%. Little is known about the number, type, and size of fibers in the cardiac branches. However the evidence from the histological studies of Daly & Evans and Agostoni et al. suggests that the number of myelinated efferent fibers to the heart is very small. This histological finding agrees well with the physiological evidence by Middleton et al. (1950) quoted earlier. On the other hand, according to Agostoni et al., the cardiac branches contain approximately 500 unmedullated efferent fibers. As there is some evidence (Heinbecker & Bishop, 1935; Koepchen et al., 1961) suggesting that unmedullated fibers may effect some cardiac slowing we cannot exclude that some of these fibers may indeed be cardioinhibitory. Another possibility to be considered is that these unmedullated fibers may be motor to the coronary arteries.

C. Recordings from cardiac vagal efferents.

While recordings from vagal afferents are numerous, recordings from vagal efferents, especially from "single" fibers, are rare.

Rjilant (1936) recorded from whole cardiac nerves of dogs after section of the sympathetic nerves and showed that, during expiration and while the slowing of the heart which appears in sinus arrhythmia was present, there was an increased rate of discharge of the middle cardiac nerves; he concluded that vagal fibers had a tonic effect on the heart which was inhibited by inspiration. Schaefer (1950) in a lengthy paper on cardiac nerves described activity recorded from cardiac nerves in the cat and claimed that activity of vagal and sympathetic bundles could be differentiated by recording from very close to their origin from the main nerve trunks. He described three types of vagal activity: continuous without periodicity and uniform over a long period of time; in groups of impulses synchronous with the pulse; and in rhythmic groups of impulses independent of the pulse. The third type of discharge became continuous during asphyxia and coincided with a slowing of the heart; furthermore drugs and physiological manoeuvres which increased arterial pressure tended to increase the discharge of these fibers. Marguth, Raule & Schaefer (1951) presented an additional account of vagal efferent activity in the cardiac nerves of the cat. The

presence or absence of bradycardia following electrical stimulation was used to distinguish between vagal and sympathetic bundles. Increased activity synchronous with bradycardia was described following injection of the sympathomimetic drug Veritol, during asphyxia and in the dying animal. Yastrebetsova & Udelnov (1955a,b,c) and Udelnov (1961) presented some dubious interpretations of results obtained from vagal branches to the frog's heart, in support of a revolutionary hypothesis on the neural regulation of heart rate. Their hypothesis proposes that the type of response of the heart (either increase or decrease in rate) depends on the number of vagal efferent fibers active at any particular time: a small number of small amplitude discharges in the cardiac efferents produces cardiac acceleration whereas a large number of large amplitude discharges produces slowing of the heart. Bonvallet & Sigg (1958) recorded from the intracranial rootlets of the vagus and separated afferent and efferent activity by sectioning the rootlets and recording from either of the two cut ends. They recorded spontaneous efferent activity of three types: synchronous with respiration even when artificial ventilation was interrupted, random activity, and discrete bursts of activity without any rhythmicity. Green (1959) recorded from multifiber preparations of the cardiac vagal branches of the cat and detected two types of vagal efferent activity: activity which appeared immediately following

baroreceptor stimulation and was not accompanied by bradycardia, and activity of the baroreceptor type in fibers presumably originating from the carotid sinus area and following an aberrant pathway to the medulla. Bianconi & Raschi (1959) confirmed Green's findings by recording rhythmic activity synchronous with the arterial pulse from the cut peripheral end of the vagus of the cat; the activity was abolished by clamping the carotid artery below the bifurcation.

Since the beginning of the work presented here four reports concerned with recording of vagal activity have appeared in the literature.

Okada, Okamoto & Nisida (1961a) described activity in one efferent vagal fiber to the heart in one cat. The frequency of discharge of this fiber increased after a small amount of water was poured into the pharynx of the animal and then decreased during deglutition.

Okada, Okamoto & Nisida (1961b) in the course of a study of the Bainbridge reflex in the cat recorded activity from "single" fibers in the cardiac branches of the vagus, after the homolateral stellate ganglion had been removed. They recorded trains of impulses synchronous with the heart beat which increased their frequency of discharge to a maximum of 120/sec or 26/train following injection of saline into the external jugular vein. This increase in activity was unchanged after cutting the sinus and aortic nerves but was abolished by contralateral

vagotomy. No mention was made of changes in heart rate during the increased frequency of discharge of these fibers, but it was suggested that, in their experiments, the Bainbridge reflex was accompanied by cardiac slowing.

Weidinger, Hetzel & Schaefer (1962) recorded efferent activity from 81 small bundles from cardiac branches in 48 cats. The activity was of three types: discharges synchronous with inspiration, which disappeared during asphyxia; discharges synchronous with the heart beat which gradually decreased their firing rate with an increase in arterial pressure following adrenaline injection; and continuous discharges which showed equivocal responses following adrenaline injection. Their criterion for identifying the vagal cardiac branches was that the recording was obtained from bundles in close proximity to the main vagal trunk.

Jewett (1962) recorded from 40 "single" efferent fibers and 15 small multifiber preparations from the cervical and cardiac branches of the vagus of dogs. The activity of these fibers was synchronous with the arterial pulse, increased at the time of cardiac slowing either spontaneously during expiration or following various experimental manoeuvres which produced bradycardia and decreased during inspiration and following experimental manoeuvres which produced tachycardia.

The physiological characteristics of efferent activity claimed to be cardiomotor and recorded in cardiac branches of the vagus are summarized in Table II.

From this brief review many discrepancies are apparent and it may be useful to examine the available data critically. It should be first pointed out that in the cat the sympathetic innervation of the heart is derived from the stellate and inferior cervical ganglia, which send fibers either directly to the heart or into the vagus trunk from which they finally reach the heart in the vagal cardiac branches (Cannon, Lewis & Britton, 1926; Anufriew, 1928). Fig. 2 shows a diagram of the autonomic innervation of the heart. This anatomical arrangement provides a mixing of sympathetic and vagal fibers which makes the interpretation of recordings of vagal activity in the cardiac branches extremely uncertain, unless the connections between vagus and sympathetic have been cut. Furthermore it is ordinarily assumed that all efferent vagal fibers to the heart are inhibitory to the sinoatrial and atrioventricular nodes. This may not be true, as evidence has been accumulating over the years indicating that the coronary arteries possess a rich motor innervation, some of which is considered to be parasympathetic (Woolard, 1926; Nonidez, 1939; Abraham, 1962).

TABLE II
EFFERENT ACTIVITY IN THE CARDIAC BRANCHES
OF THE VAGUS

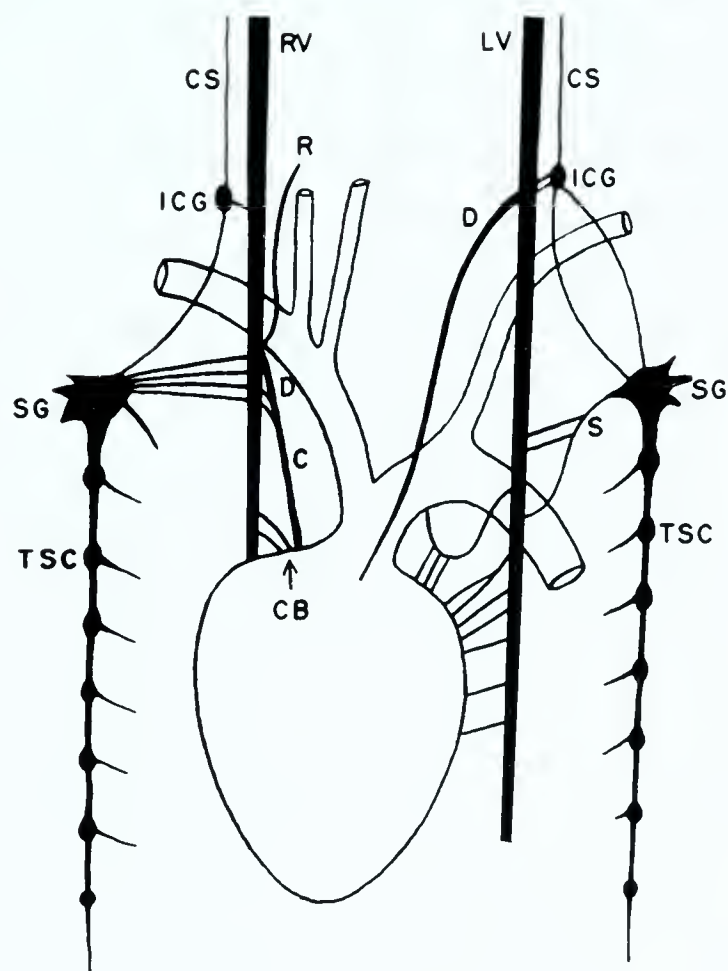
Authors and year	Species	Identification of vagal branches	Spontaneous activity	Activity following stimuli
Rjilant (1936)	dog	section of sympathetic nerves	increased during expiration; decreased during inspiration	
Schaefer (1950)	cat	none mentioned	random continuous	
			cardiac rhythm	
			rhythmic groups	increased & continuous during asphyxia and hypertension
Marguth <u>et al.</u> (1951)	cat	bradycardia following electrical stimulation	random	increased in the dying animal, during asphyxia, hypertension and bradycardia
Green (1959)	cat	none mentioned	random	increased during baroreceptor stimulation
Okada <u>et al.</u> (1961a)	cat	stellate ganglion removed	random	decreased during deglutition
Okada <u>et al.</u> (1961b)	cat	stellate ganglion removed	cardiac rhythm	increased following saline injection into external jugular vien

...../continued

TABLE II - Continued

EFFERENT ACTIVITY IN THE CARDIAC BRANCHES
OF THE VAGUS

Authors and year	Species	Identification of vagal branches	Spontaneous activity	Activity following stimuli
<u>Weidinger et al.</u> (1962)	cat	recording from bundles close to vagus trunk	inspiratory rhythm	absent during asphyxia
			cardiac rhythm	decreased during hypertension
			continuous	equivocal during hypertension
Jewett (1962)	dog	none mentioned	cardiac rhythm, increased during expiration and decreased during inspiration	decreased during tachycardia, increased during bradycardia



C = "COMMON CARDIAC N."	R = RECURRENT LARYNGEAL N.
CB = CARDIAC BRANCHES	RV = RIGHT VAGUS
CS = CERVICAL SYMPATHETIC	S = SYMPATHETIC BUNDLE
D = DEPRESSOR N.	SG = STELLATE GANGLION
ICG = INF. CERVICAL GANGLION	TSC = THORACIC SYMP. CHAIN
LV = LEFT VAGUS	

Fig. 2. Autonomic innervation of the heart in the cat (modified from Cannon et al., 1926).

From these considerations it follows that in order to identify parasympathetic inhibitory activity in the cardiac branches of the vagus it is necessary to sever all connections between sympathetic nerves and ganglia and the vagus nerve. In addition, it should be remembered that some of the activity recorded from cardiac branches may be destined to the coronary arteries.

In none of the experiments reviewed here has it been demonstrated that all connections between the vagus and the sympathetic had been severed. It is quite possible, then, that some of the recordings were obtained from sympathetic fibers. For instance, the changes in activity observed by Okada et al. (1961 b) are those one would expect to find in sympathetic cardiac fibers during the Bainbridge reflex. The technique used by Marguth et al. is also open to criticism because the fact that electrical stimulation of a nerve bundle produces a certain effect (in this case, bradycardia) is no proof that the bundle is composed of homogeneous fibers. A final remark should be made about Jewett's work. His results have not been published in a full length paper and there are no records available. The description of the activity exhibited by the fibers he studied is entirely consistent with the expected behavior of cardioinhibitory fibers, and if proof of complete separation of vagal and sympathetic nerves is presented in the future, his results will have to be accepted.

D. Histology of the dorsal nucleus of the vagus

The dorsal nucleus of the vagus is located in the medulla oblongata. In the cat it extends rostro-caudally for approximately 4 mm, caudally lies lateral and dorsal to the central canal, and rostrally lies ventral to the floor of the fourth ventricle. Cells of three sizes can be differentiated within it: large, intermediate, and small. Fig. 3 shows a longitudinal section of the cat medulla which includes part of the dorsal nucleus of the vagus. Figure 4 shows the outline in three planes of the dorsal nucleus, reconstructed from serial cross sections of the medulla of one cat.

According to Mitchell & Warwick (1955) Stilling (1843) is credited with the first description of the dorsal nucleus of the vagus. According to Ramon y Cajal (1911) the dorsal nucleus, on the basis of Koelliker's work, was believed to be a sensory nucleus of the ninth and tenth cranial nerves until the work of Forel and Marinesco showed that retrograde degeneration within the nucleus could be demonstrated following section of the vagus nerve. Ramon y Cajal (1911) agreed with the view that the dorsal nucleus was a motor nucleus.

Molhant (1910) described the dorsal nucleus in the rabbit and in an extensive series of experiments showed that section of the bulbar rootlets

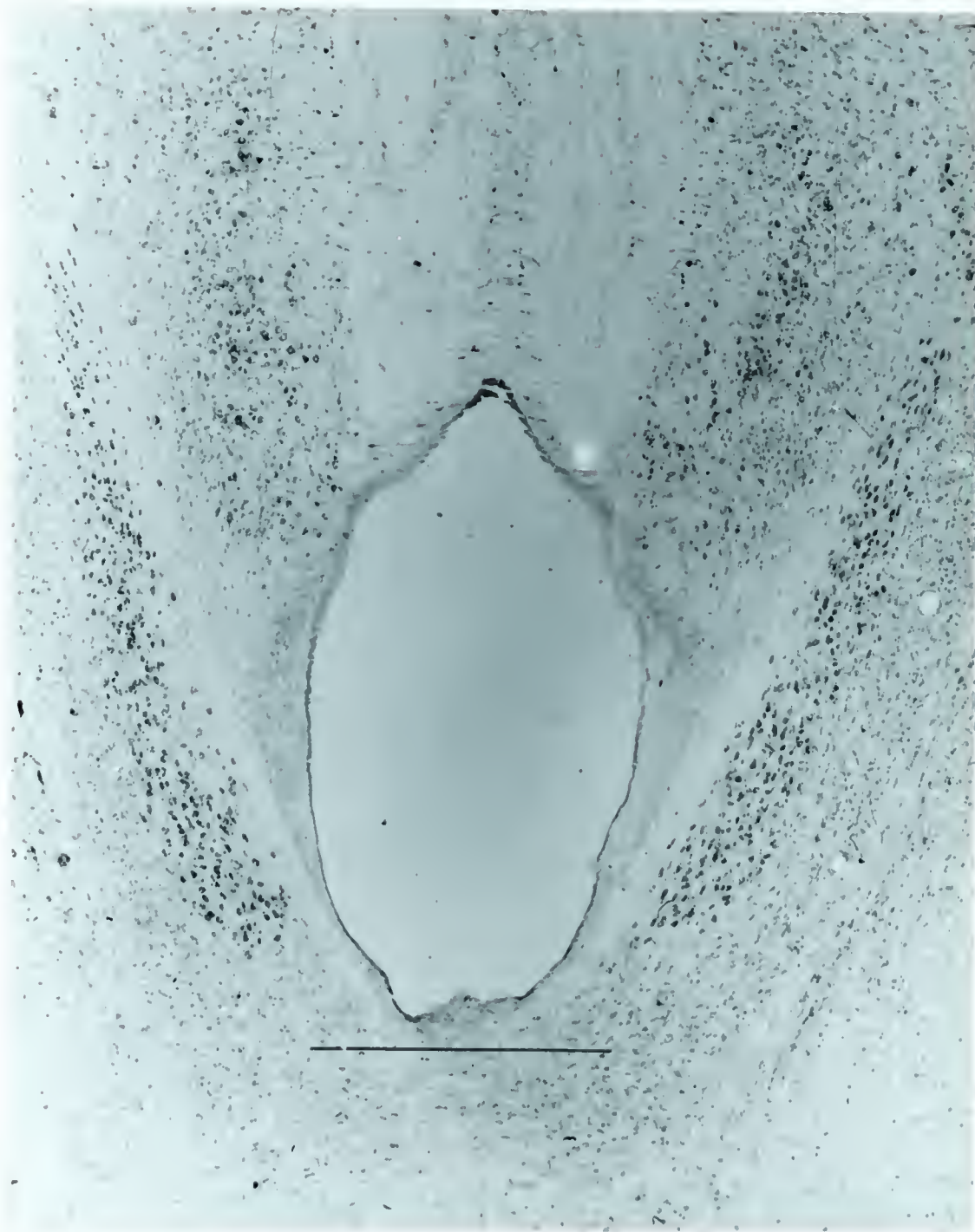


Fig. 3. Longitudinal section of the medulla of a cat showing part of the dorsal nucleus of the vagus.
Calibration 1 mm.

OUTLINE OF DORSAL NUCLEUS OF THE VAGUS AND
HYPOGLOSSAL NUCLEUS IN A CAT

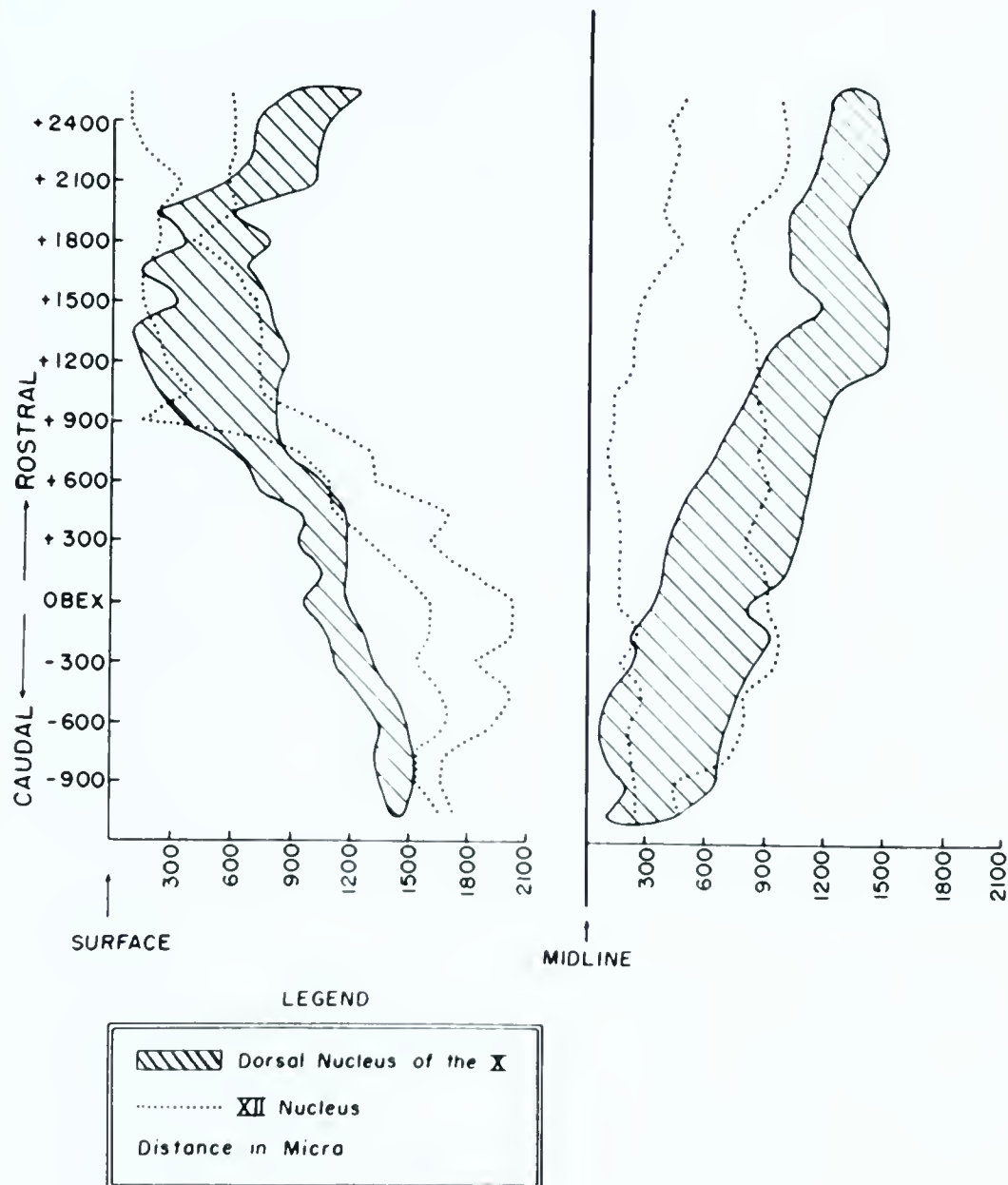


Fig. 4. Outlines of the right dorsal nucleus of the vagus and the hypoglossal nucleus reconstructed from serial sections of the medulla of one cat. On the left : lateral projection of the rostro-caudal and dorso-ventral extents of the nucleus. On the right : surface projection of the rostro-caudal and lateral extents of the nucleus. Distances in μ .

of the vagus and the accessory produced retrograde degeneration within the nucleus; on the other hand, section of the rootlets of the glossopharyngeal failed to produce any changes within the nucleus. Furthermore, section of the superior cardiac branches of the vagus produced a small diffuse amount of retrograde degeneration in the middle third of the dorsal nucleus but did not produce degeneration within the nucleus ambiguus and section of different branches of the vagus produced retrograde degeneration in fairly well defined areas within the nucleus. He therefore concluded that the dorsal nucleus of the vagus was the motor nucleus of the smooth muscles of the viscera. In addition, Molhant tried to produce lesions of the nucleus and study the ensuing Wallerian degeneration in the branches of the vagus and the functional changes in the animals so operated upon. He succeeded in producing a partial lesion of the nucleus in one rabbit. Degeneration was observed in the ipsilateral vagus trunk; however stimulation of the ipsilateral cervical vagus in this animal still produced cardiac slowing: he explained this result by suggesting that the lesion of the dorsal nucleus probably involved too few cardioinhibitory cells. Malone (1913) studied the dorsal nucleus in one lemur and one macacus rhesus and described two types of cells; small cells rostrally and caudally and larger ones in the intermediate region.

On the basis of their morphology he suggested that the small cells were motor to the smooth visceral muscles and the larger ones were motor to the heart and he designated the intermediate area of the dorsal nucleus as the "nucleus cardiacus nervi vagi". Getz & Sirnes (1949) used seven rabbits to study retrograde cell changes in the dorsal nucleus following section of the vagus nerve at four different levels and concluded that the functional representation within the nucleus from the rostral to the caudal end was: lungs and possibly bronchi, abdominal organs, heart and esophagus, trachea and possibly bronchi. They also showed that when the vagus nerve was cut proximal to the superior laryngeal nerve no normal cells were found within the nucleus, which seems to indicate that the dorsal nucleus is connected exclusively with motor fibers of the vagus nerve. Szentagothai (1952) placed small electrolytic lesions in different parts of the brain-stem of cats and looked for signs of degeneration in peripheral vagal fibers. After placing small lesions in the dorsal nucleus and after ablating the whole ala cinerea he failed to observe any degenerated fibers in the peripheral vagus and concluded:

""...no preganglionic fibers at all originate from the so called "dorsal vagal nucleus".""

Furthermore, following lesions of the nucleus ambiguus he detected signs of degeneration in the cardiac and especially in the pulmonary branches of the vagus and

concluded:

"...from a large number of experiments the impression is gained that preganglionic fibers arise especially from dorsolateral parts of the nucleus ambiguus, but exclusively from the oral half."

Mohiuddin (1953) studied retrograde degeneration in the dorsal nucleus and Wallerian degeneration in the vagus of kittens and cats following section of the vagus at different levels. He described the cells within the nucleus as medium size (25-30 micra in diameter) or small. By counting the number of nucleoli in serial sections he concluded that the dorsal nucleus contained approximately 6,000 cells of medium size. He failed to detect any retrograde changes in the small cells after section of the vagus; with regard to the larger cells he concluded:

"One may state tentatively that about half the large cells in the dorsal nucleus of the vagus show degenerative changes following section of the vagus in the thorax, distal to the origin of the pulmonary branches."

Mitchell & Warwick (1955) carried out an extensive study of the dorsal nucleus in 45 rhesus monkeys. They studied retrograde degeneration in the nucleus following section of the vagus at different levels. Their conclusions are summarized.

(a) Definite chromatolytic changes were found in the dorsal nucleus following section of the vagus at all levels.

(b) The small cells of the nucleus showed no or only equivocal changes following section of the vagus; this implies that they were probably sensory or possibly interneuronal.

(c) Some chromatolytic changes were detected in the dorsal nucleus following section of the ninth and eleventh nerves.

(d) Some chromatolytic changes were detected on the side opposite the lesion especially when the vagi were divided at lower levels; this implies the presence of a minor degree of decussation.

(e) Functional representation was incompletely inverted, and specifically section of the cardiac plexus below the aortic arch produced retrograde changes in many cells of the middle third of the nucleus and in a small number of cells in the upper and lower thirds.

(f) Three different types of cells were distinguished in the nucleus: small, medium and large. The small cells were more numerous on the lateral border of the nucleus; the medium size cells were pyramidal or fusiform in shape; and the large cells were multipolar, were present in all regions of the nucleus but appeared to be more numerous in the intermediate third.

Kitchell, Stromberg & Davis (1956)
transected the vagus nerve at various levels in 14 calves, 14 sheep, 2 horses, one dog, one cat, one goat and one pig and studied retrograde degeneration in the dorsal nucleus. They concluded that the dorsal

nucleus of ruminants contained a higher number of cells than that of non-ruminants and that the representation of abdominal organs was mainly in the rostral part and that of thoracic organs in the caudal part of the nucleus. Schultz (1959) in 13 human medullae from cases of lesions of the esophagus and stomach described retrograde degeneration within the dorsal nucleus of the vagus, but was unable to show a topographical organization. Watanabe & Yasuda (1960) sectioned the vagus nerve in fowl at the level of the stomach and showed retrograde degeneration in the middle third of the dorsal nucleus. Bell (1960) studied retrograde degeneration in both dorsal nuclei of goats and sheep following section of one vagus. He concluded that division of the cervical vagus produced degeneration of almost all the cells within the ipsilateral nucleus and no changes in the contralateral one, and section of the abdominal vagus produced degeneration in both nuclei; the latter finding was attributed to the peripheral mixing of branches from the two sides; he also observed that thoracic viscera had a diffuse representation but were mainly represented in the caudal half, and abdominal viscera were almost exclusively represented in the rostral half.

Taber (1961) in a comprehensive study of the brainstem nuclei of the cat described the "nucleus nervi vagi dorsalis motorius" as follows:

"The extent of this ovoid-shaped nucleus (circa 4.2 mm) slightly exceeds the caudo-rostral length of the nucleus nervi hypoglossi. Caudally it lies lateral to the central canal, while rostrally it is located ventral to the floor of the 4th ventricle. The nucleus is composed of small, intermediate and medium-sized cells. The small cells are oval or almost round each with a relatively large nucleus, a scarce amount of cytoplasm and clumped Nissl substance. The number of these cells is greater in the antero-ventral portion of the nucleus. The intermediate-sized cells are fusiform or pyramidal-shaped, each with a central nucleus and scattered Nissl substance. These cells are more abundant in the mid-extent of the nucleus. The medium-sized cells are multipolar, each with an eccentric nucleus and large, irregular Nissl body masses concentrated at the cell membrane. These latter cells stain intensely, have a "ragged" appearance and are dispersed throughout the nucleus."

Nishi (1962) described chromatolytic changes in 70% of the cells of the dorsal nucleus of the mouse following section of the cervical vagus.

The results of histological studies on the dorsal nucleus are summarized in Table III.

In summary, all the available evidence from studies of retrograde degeneration seems to indicate that the cells of medium and intermediate size in the dorsal nucleus are visceral motor cells of the tenth cranial nerve, that each nucleus is mainly connected with the ipsilateral vagus and that the nucleus has a sensory component. With regard to the sensory component, it is very unlikely that these small cells are second order afferent neurones as suggested by Mitchell & Warwick

TABLE III
HISTOLOGICAL STUDIES ON THE DORSAL NUCLEUS
OF THE VAGUS

Authors and year	Species and technique	Function	Location of cardiomotor cells
Molhant (1910)	rabbit retrograde degen.	motor	middle third
Getz & Sirnes (1949)	rabbit retrograde degen.	motor	middle third
Szentagothai (1952)	cat ablation of nucleus	no connections with the vagus	
Mohiuddin (1953)	cat retrograde degen.	motor	
Mitchell & Warwick (1955)	rhesus monkey	motor and sensory	primarily middle third
Kitchell <u>et al.</u> (1956)	cat, sheep, horse, dog, calf, goat, pig retrograde degen.	motor	caudal half
Schultz (1959)	man retrograde degen.	motor	
Watanabe & Yasuda (1960)	fowl retrograde degen.	motor	
Bell (1960)	goat & sheep retrograde degen.	motor	caudal half
Nishi (1962)	mouse retrograde degen.	motor	

(1955), in view of the absence of terminal degeneration around these cells following supranodose vagotomy (Cottle, in the Press); however they may be interneurons in a polysynaptic pathway regulating visceral activity. In addition the findings of three separate groups (Molhant; Getz & Sirnes; Mitchell & Warwick) seem to indicate that the cell bodies of the cardiomotor fibers are located approximately in the middle third of the nucleus. In contrast with the studies of retrograde degeneration the work of Szentagothai indicates that the dorsal nucleus is not the visceral motor nucleus of the vagus. Even though Szentagothai's results cannot be compared to those obtained by the retrograde degeneration technique, it seems unlikely that ablation of a nucleus which shows retrograde degeneration when its axons are cut, would not produce Wallerian degeneration in its axons. Szentagothai's results must be considered with caution because the location and the extent of the lesions he produced in his experimental animals are not shown in his paper. Furthermore Molhant, who gives proof of being a more careful experimenter, was able to show Wallerian degeneration in the vagus nerve of the animal in which he had produced a lesion of the dorsal nucleus.

E. Neurophysiology of the dorsal motor nucleus of the vagus.

The Weber brothers (1845) were the first to observe

that electrical stimulation of the medulla oblongata of the frog produced slowing of the heart. A facsimile of their interesting paper is shown in Fig. 5.

Laborde (1888) made the first attempt to localize a cardioinhibitory center in the medulla. In reviewing the evidence available at that time he stated (my translation):

"The famous experiment carried out, at the same time, in Germany, by Budge and the Weber brothers and by Claude Bernard in France, in which an induction current, passing through the medulla, determines instantly a slowing of the heart beat and a diastolic relaxation, was without a doubt of a nature to make it possible to predict the existence of a central region with particular influence on heart movements."

Furthermore he claimed that he could produce bradycardia in cats by stimulating by "piqûre" through the floor of the fourth ventricle a "point lateral and a little below the mixed columns ... in the direction of the inferior third of the restiform bodies", and an area quite deep in the medulla, to which he refers as "motor nuclei of the bulbar nerves" and which may correspond roughly to the nucleus ambiguus.

Miller & Bowman (1916) produced bradycardia in dogs by faradic stimulation of the ala cinerea on the floor of the fourth ventricle. No bradycardia was obtained by stimulating neighboring areas. Laughton (1929) produced both excitation and inhibition of gastric motility in cats by faradic stimulation of the floor of the fourth ventricle in an area corresponding

II. Eduardi et Ernesti Henrici Weberi EXPERIMENTA, QUIBUS PROBATUR NERVOS VAGOS ROTATIONE MACHINE GALVANO-MAGNETICÆ IRRITATOS, MOTUM CORDIS RETARDARE ET ADEO INTERCIPERE.

1. Si medullam oblongatam ranæ aut fines nervorum vagorum ibi resectorum rotatione machinæ galvanomagneticæ fortioris irritaveris, cor repente motu privatur, at vero finita irritatione, brevi temporis spatio elapso, denuo pulsare incipit, initio tarde nec totum, sensim paulatimque fortius et frequentius, ita ut tandem pristinus motus ante irritationem observatus restituatur.

2. Rotatio machinæ non satis fortis motum cordis retardat et infirmit. Cor, cujus motus hac ratione intercipitur nequaquam contractione tetanica constringitur, sed laxum est planamque formam habet.

3. Irritatio nervi vagi in uno latere positi motum cordis non mutat.

Si irritatio nervorum vagorum tam diu continuatur, ut vis eorum irritationes propagandi exauriatur, cor denuo pulsare incipit.

4. Partibus cordi vicinis, in quibus nervi sympathici decurrunt aut ramos dispergunt, eodem modo irritatis, cor motu neque privatur, neque retardatur, pulsationes ejus potius frequentiores redduntur, et si motus cordis antea sublatus erat, adeo restituitur.

Incertum vero est, num hic effectus magis a nervo sympathico irritato quam ab electricitate per materiam animaleam humidam ad cor immediate derivata pendeat.

5. Fila metallica apparatus galvanomagnetici si immediate ad cor certo quodam modo applicaveris, accidere potest, ut cor tetanica contractione constringatur et tamdiu motu privetur, quamdiu constrictio illa durat.

6. Irritatio nervorum vagorum descripta similem effectum in cuniculis quam in ranis habet.

Fig. 5. Facsimile of the paper by the Weber brothers (Annali Universali di Medicina 20:227, 1845): first description of bradycardia caused by electrical stimulation of the medulla and peripheral vagus in frogs and rabbits.

to the surface projection of the rostral end of the dorsal nucleus of the vagus. Larson (1954) showed that stimulation through permanently implanted electrodes of the dorsal nucleus of the goat produced an increase in food intake. Anderson & Berry (1956) using large electrodes recorded evoked potentials in the medulla of cats, following electrical stimulation of the aortic depressor and vagus nerves. In particular they claimed they recorded antidromic potentials in the dorsal nucleus of the vagus and in the nucleus ambiguus, but their localization of the sites of recording was rather unrefined. Clement, Sutin & Silverstone (1957), using large electrodes (tip 20-50 micra) detected increased electrical activity in the region of the dorsal nucleus following intravenous or intra-arterial injection of hypertonic Na Cl solution. Stefantsov (1958) removed surgically the left dorsal nucleus and the surrounding area in 36 dogs. In the 12 dogs that survived, tachycardia, tachypnea, disturbances in swallowing, constipation, retention of urine, contralateral reduction of skin temperature and difficulty in standing and walking were present four days after the operation; there was a complete return to normal within one month.

Urabe & Tsubokawa (1960) carried out an extensive series of experiments in 62 cats; they stimulated electrically the peripheral vagus either

in the neck or within the chest and attempted to record evoked potentials in the homolateral medulla. Silver electrodes with a tip 5 to 10 micra in diameter were used and the site of recording was marked with small electrolytic lesions. They were able to record evoked potentials from various nuclear masses within the medulla, but they failed to do so from the dorsal nucleus. Conduction velocities of the recorded impulses were between 18 and 110 meters per second. Furthermore when the vagus nerve was stimulated in the thoracic cavity "evoked potentials could be recorded only in the vicinity of the solitary tract and triangular nucleus of the vestibular nerve."

Sperti & Xamin (1960) stimulated electrically "a rather large medullary area including the dorsal motor nucleus of the vagus" in 21 dogs and produced bradycardia, which was still present when the stimulation of the medulla was repeated after section of the homolateral vagal roots but was completely abolished by section of the homolateral accessory root. The sites of stimulation were not identified histologically.

Kovalev & Bondarev (1962) in a large series of experiments in 194 decerebrate cats stimulated electrically different areas of the medulla and recorded heart rate and arterial pressure changes. The electrodes used were nichrome wires 30 to 50 micra in diameter

and histological localization was obtained by placing small electrolytic lesions. In twenty cases in which the dorsal nucleus was stimulated the response was a fall in arterial pressure in 18 and an increase in 2. It is not clear what the changes in heart rate were on these occasions but the authors state that on some occasions the "depressor reaction (i.e., hypotension) was accompanied by a slowing of the heart rate."

Rudomin (1963) in a recent abstract claimed that stimulation of the dorsal nucleus of the vagus in the cat (histological verification was claimed) produced an electrical response in the ipsilateral vagus with a latency from 0.6 to 2.0 msec, and that the structures stimulated could be driven to frequencies above 200 per second. No mention was made of the physiological responses following stimulation.

Porter (1963) in a series of 32 cats attempted to record evoked mass responses and unit potentials from the medullary nuclei of the vagus following electrical stimulation of the peripheral vagus and its branches. The site of recording was verified histologically. He was not able to record any responses in the dorsal nucleus, but recorded evoked potentials with a latency between 1 and 20 msec from the nucleus of the tractus solitarius.

The results of physiological experiments on the dorsal nucleus are summarized in Table IV.

In summary, the stimulation experiments carried out to date have not been selective enough to justify the conclusion that stimulation of the dorsal nucleus produces bradycardia; it is quite possible that the bradycardia observed was produced by stimulation of other structures within the medulla.

With regard to the recording of evoked potentials, the positive results of Anderson & Berry should be regarded with caution because of lack of histological localization. However, the negative results of Urabe & Tsubokawa and of Porter should be accepted because the sites of stimulation were identified histologically and because convincing evidence was presented. This failure to record evoked potentials could be explained by postulating the existence of a synapse between dorsal nucleus and peripheral vagus: obviously if a synapse were present in the vagal efferent pathway only orthodromic stimuli could get through. The possibility of the existence of such a synapse intercalated in the vagal motor pathway must be considered in view of Hoffman & Kuntz's description of ganglion cells within the trunk of the vagus both in humans and cats. However, in view of the wealth of histological evidence from retrograde degeneration studies, the failure of the evoked potential experiments

TABLE IV
PHYSIOLOGICAL STUDIES ON THE DORSAL NUCLEUS
OF THE VAGUS

Authors and year	Species	Technique	Effects
Weber brothers (1845)	frog and rabbit	electrical stimulation of medulla	bradycardia
Laborde (1888)	cat	piqûre of medulla	bradycardia
Miller & Bowman (1916)	dog	faradic stimulation of ala cinerea	bradycardia
Laughton (1929)	cat	faradic stimulation of dorsal nucleus	excitation and inhibition of gastric motility
Larson (1954)	goat	electrical stimulation of dorsal nucleus	increase in food intake
Anderson & Berry (1956)	cat	electrical stimulation of vagus and depressor nerves	evoked potentials in dorsal nucleus
Clemente <u>et al.</u> (1957)	cat	recording from dorsal nucleus	increased activity after injection of hypertonic saline
Stefantsov (1958)	dog	ablation of dorsal nucleus	tachycardia and other disturbances
Urabe & Tsubokawa (1960)	cat	electrical stimulation of vagus nerve	no evoked potentials in dorsal nucleus
Sperti & Xamin (1960)	dog	electrical stimulation of medulla including dorsal nucleus	bradycardia

...../continued

TABLE IV - Continued

PHYSIOLOGICAL STUDIES ON THE DORSAL NUCLEUS
OF THE VAGUS

Authors and Year	Species	Technique	Effects
Kovalev & Bondarev (1962)	cat	electrical stimulation of dorsal nucleus	hypertension or hypotension, occasionally bradycardia
Rudomin (1963)	cat	electrical stimulation of dorsal nucleus	action potential in cervical vagus
Porter (1963)	cat	electrical stimulation of vagus nerve and its branches	no evoked potentials in dorsal nucleus

is hard to explain, unless the retrograde degeneration seen in the dorsal nucleus, following section of the vagus nerve, is a special type of transneuronal degeneration never described before, in a direction opposite to that of physiological impulse traffic.

With regard to Rudomin's work, his findings seem to provide electrophysiological evidence for a direct link between the dorsal nucleus and peripheral vagus, but until more data are made available about the method of histological localization it would be wise to regard his conclusions with caution. For example, from his data on latencies the conclusion can be drawn that there are vagal efferent fibers conducting at a velocity of roughly 100 meters per second, which, in comparison with values in the literature, seems to be an extraordinarily high figure. It is quite possible then, that the evoked potentials in the vagus were obtained by stimulating the tractus solitarius or its nucleus in locations where these two structures are very close to the dorsal nucleus.

To conclude, it appears impossible to draw any firm conclusions from the physiological experiments and obviously more and better experiments are needed to clarify the problem of connections between dorsal nucleus and peripheral vagus.

F. Conclusion and Statement of Objects of Research

It may be useful, at this point, to try to draw conclusions about the available data and propose experiments designed to answer some of the questions arising from the review of the literature. The experiments reviewed have already been examined critically under the separate headings and an integration of the knowledge obtained from data which have survived critical analysis will now be attempted.

It can be concluded that cardioinhibitory fibers in the cat travel in the vagus nerve, their number is probably small, they can be classified as myelinated fibers of small diameter and, according to the more reliable data by Koepchen et al., have a conduction velocity of roughly 5 meters per second. Furthermore it should be kept in mind that some cardiomotor fibers may belong to the unmyelinated group. Unfortunately, there are no reliable data on the activity of vagal cardiomotor fibers because none of the eight reports published can be accepted as the identity of the fibers has not been demonstrated.

All the studies of retrograde degeneration of cells in the dorsal nucleus have established a direct connection between dorsal nucleus and vagus nerve; furthermore, in three studies it has been suggested that the cell bodies of the cardiac fibers are located in the middle third of the nucleus. Szentagothai's

claim that following destruction of the dorsal nucleus no Wallerian degeneration could be demonstrated in vagal fibers should be considered with caution because he presented no convincing evidence. The inability to record evoked potentials from the dorsal nucleus, in view of the histological findings, remains a puzzling observation for which no ready explanation can be offered. Finally evidence that selective stimulation of the dorsal nucleus produces bradycardia is lacking.

To find the answer to some of the questions raised by the study of the literature several experimental approaches may be suggested. Of those considered the following four were selected.

Firstly, attempts should be made to record spontaneous activity as well as that following stimuli known to produce reflex changes in heart rate, from efferent fibers in the cervical vagus and in cardiac branches, after severing all sympathetic connections with the vagus. In addition, if single efferent fibers can be isolated, attempts should be made to measure conduction velocity in a manner more direct than that of previous studies.

Secondly, attempts should be made to record from single units in the dorsal nucleus and to investigate whether they change their activity in time with reflex changes in heart rate.

Thirdly, discrete lesions of the medulla should be produced in the attempt to damage the dorsal nucleus and to study the accompanying Wallerian degeneration in the vagal efferents.

Fourthly, selective electrical stimulation of the dorsal nucleus of the vagus should be carried out in order to establish the effect of its stimulation on the heart rate. At the same time the electrical activity of the ipsilateral vagus nerve should be recorded, in an attempt to correlate cardioinhibitory effects and components of the compound action potential of the vagus and to obtain an approximate measurement of conduction velocity in cardioinhibitory fibers.

METHODS

Four groups of cats were used. The methods used in each group are described separately.

A. Recordings from vagal efferent fibers.

38 adult cats anesthetized with Chloralose (65 mg/kg intravenously) after ethyl chloride and ether induction were used. A tracheal cannula was inserted and the right saphenous vein was cannulated and used for drug injection. The rectal temperature of the animal was kept between 37° and 39°C throughout the experiment. The physiological manoeuvres and drugs used to produce cardiovascular responses were carotid occlusion, below or above the carotid sinus, and

intravenous administration of 50 to 300 micrograms of phenyldiguanide (PDG). This is one of a series of amidine derivatives known to cause marked hypotension and bradycardia with a transient inhibition of respiration in the intact but not in the vagotomized animal (Dawes, Mott & Widdicombe, 1951).

(1) "Single" fiber technique. This technique was used in 28 cats of this series. The cervical vagus nerve, usually the right, was exposed, freed for a distance of roughly 50 mm and immersed in a pool of paraffin oil formed by a suitable arrangement of the skin and muscular layers of the wound in the neck. Small nerve strands were dissected free from the vagus, cut peripherally and placed on a pair of recording silver electrodes. The fine dissection of small slips of nerve was carried out with the aid of a binocular dissecting microscope. The electrical activity of "single" fibers was fed to a conventional amplifying and recording apparatus composed of a P-5 Grass differential preamplifier, one beam of a Nagard DT 103 cathode ray oscilloscope and a Dumont 321-A oscilloscope camera. Ilford NS6 recording paper was used. An audio amplifying system was connected in parallel with the nerve channel to monitor the electrical activity. The electrical activity of the heart, after amplification through a P-8 Grass preamplifier, was displayed on the other beam of the oscilloscope.

This method gave consistently satisfactory results. An example of recorded activity is shown in Figure 6.

(2) "Collision technique". This technique, originally described by Douglas & Ritchie (1957), was used in 10 cats of this series. The whole cervical vagus, freed from surrounding connective tissue for as long a distance as possible and cut distally, was placed on two pairs of silver electrodes: one pair was used for electrical stimulation and a similar pair for recording the response. A Grass S4E stimulator was used and the stimulus (square wave, 0.5-1 msec duration, 5-10 volts, frequency 50 per second) was delivered to the preparation through a Grass SIU-4 isolation unit. The electrical activity from the recording electrodes, amplified by a Grass P-5 preamplifier was fed to the Y axis of one of the beams of a Nagard DT 103 oscilloscope. Electrical activity of the heart, after amplification, was simultaneously fed to the X axis of the second beam of the same oscilloscope, after it had been disconnected from the time base generator. The electrical activity of the whole vagus and the electrocardiogram could be photographed on a vertically moving film. The same manoeuvres and drugs used in the "single" fiber experiments were used to produce cardiovascular responses.

B. Recordings from the dorsal nucleus of the vagus.

(1) Mapping of the nucleus. Before attempting the neuro-

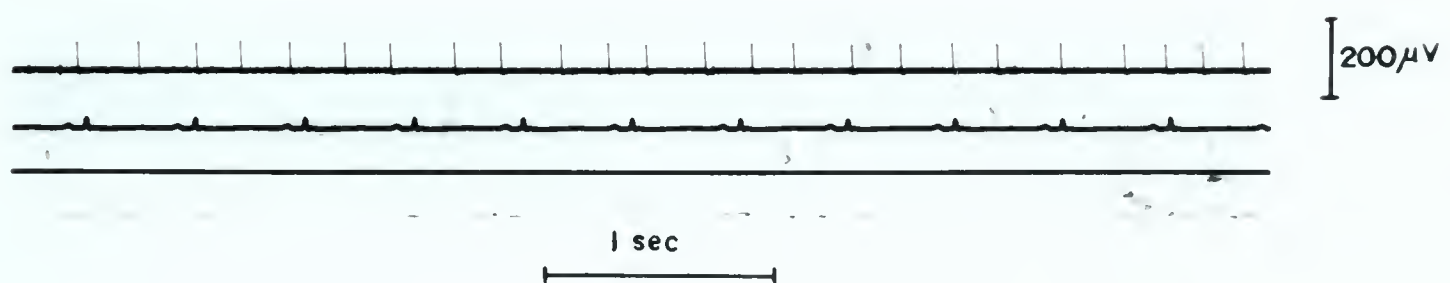


Fig. 6. Cervical vagus unit 27-3. Activity from a "single" efferent fiber.
From above downwards : electroneurogram,
electrocardiogram and event marker.

physiological study of the dorsal nucleus a tridimensional map of this structure from the study of serial sections of the medulla of one cat was obtained. 300 serial cross sections of the medulla, 15 micra thick, stained according to the Weil technique were projected through a Leitz projector (objective 1:4.5) onto a screen approximately three feet away from the objective. The outlines of the dorsal and hypoglossal nuclei together with standard landmarks, enlarged approximately 50 times, were traced on transparent paper and from the tracings the outlines shown in Fig. 4 were obtained.

(2) Physiological experiments. 51 adult cats were used. Of the 33 cats included in the results 21 were under Chloralose anesthesia (65 mg/kg intravenously) and 12 were artificially ventilated decerebrate spinal cats. A tracheal cannula and polyethylene catheters in the right saphenous vein and right femoral artery were inserted routinely. The animals were then fixed in a Johnson Scientific Instruments stereotaxic apparatus fitted with an electrode advancing micrometer. Lateral and longitudinal movements of the electrode could be effected in steps of 100 micra and vertical movements in steps of 10 micra. The occipital bone was exposed and removed and the cut edges of the bone plugged

with plasticine. The cerebellum was removed by suction to expose the floor of the fourth ventricle. In the decerebrate animals ether was administered up to the time of the post-tentorial midcollicular decerebration which was carried out by the use of a small blunt spatula, while both the carotid arteries were obstructed by applying tension to loops of thread around them. In the animals in which spinal section was to be performed, the arch of the atlas and the meningeal membranes were removed and section at C₁-C₂ level was carried out by the use of a spatula. Paraffin oil was poured over the exposed nervous structures to prevent drying and the animals' rectal temperature was kept between 37° and 39°C. In some experiments, to prevent movement of the medulla, as soon as the electrode had made contact with the floor of the fourth ventricle, a warm suspension of 3% agar in saline or warm Carbowax (melting point = 40°C) was poured over the exposed fourth ventricle.

Penetrations in locations which were most likely to be within the dorsal nucleus were carried out with stainless steel microelectrodes. They were manufactured from stainless steel insect pins, size 00, according to the procedure suggested by Green (1958); the diameter of the tip varied from 2 to 6 micra and the D.C. resistance in saline was less than one megohm. The indifferent electrode was a

hypodermic stainless steel needle which was thrust into the substance of the forebrain. The two electrodes were connected directly to the input of a Grass P-5 differential preamplifier.

The arterial pressure was measured by a Statham P 23-A pressure transducer, and through one of the channels of an E.f.M. polygraph was fed to one of the beams of the oscilloscope. The oscilloscope, the recording camera and the audio monitoring system were previously described under Section A (1).

This method gave satisfactory results: examples of recorded activity are shown in Fig. 7.

The physiological manoeuvres and drugs used to elicit cardiovascular responses were those described under Section A.

(3) Histological localization of the sites of recording.

The reference points used for positioning the electrodes were the obex and the midline. As it became apparent that an accurate correlation between neurophysiological data and anatomical localization could not be obtained by relying on stereotaxic coordinates, a technique for marking the site of recording was used (Green, 1958). Iron deposits were produced by passing a D.C. current (2-5 microamps for 2-10 seconds) through the recording electrode as the anode.

At the end of each experiment the animals were perfused with a 0.9% NaCl solution followed by a 1% solution of potassium ferrocyanide in 10% formalin.

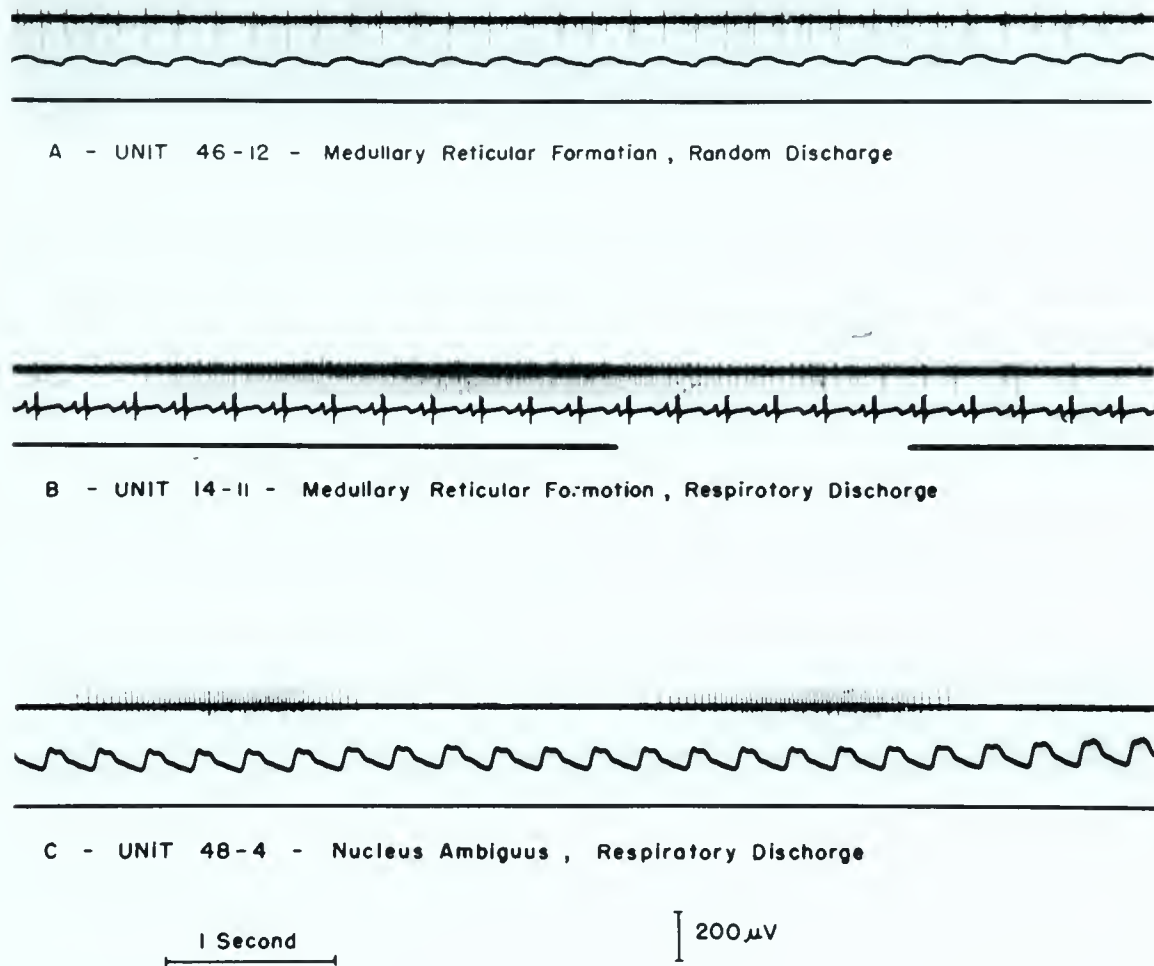


Fig. 7. Examples of electrical activity of single units in the medulla.
 In records A and C from above downwards :
 electroneurogram, arterial pressure and
 event marker.
 In record B : electroneurogram, electro-
 cardiogram and event marker (during the
 interruption beginning of inspiration).

The medulla was then removed and fixed for 5 to 10 days in a solution with the following composition : 1% potassium ferrocyanide in 10% formalin 8 parts, 2% acetic acid in 95% ethyl alcohol 2 parts. At the end of the fixation period the medulla was washed in running water for a few hours and the meningeal membranes were removed. Frozen serial sections 50 micra thick were cut on a Leitz freezing microtome, floated in water and Mayer's egg albumin (3 drops of egg albumin in 20 ml of water) and mounted on slides. After drying for an hour in an oven at 40-50° C the sections were dehydrated, cleared in two changes of an ether-chloroform mixture (1:1) for 15 minutes each, left out in the air for 15 minutes and then taken through a descending alcohol series. After staining for 3 minutes in a solution of Kernechtrot (100 mg of Kernechtrot dissolved with the aid of heat in 100 ml of a 5% solution of aluminum sulphate in water), the slides were taken through an ascending alcohol series and xylol and covered.

The sites of iron deposition appeared as blue spots of various sizes against a light red background. After calibration of a Zeiss microscope by the use of stage and ocular micrometers, measurements of location of iron deposits with respect to obex, midline and surface of the floor of the fourth ventricle were made and the position of the deposit with respect

to nuclear structures was determined. Finally a correlation between electrical recordings and histological data was obtained.

C. Lesions of the dorsal nucleus of the vagus.

(1) Surgical procedure. 17 adult cats anesthetized with sterile Nembutal (35-40 mg/kg intraperitoneally) were used. The heads of the animals were immobilized in a head holder and under aseptic conditions the right caudal part of the floor of the fourth ventricle was exposed, after removal of part of the occipital bone, hemostasis of the bone edges by the use of Horsley's bone wax and removal of the caudal part of the vermis cerebelli and part of the right cerebellar lobe. A lesion was produced in a small area of the right ala cinerea where the middle third of the dorsal nucleus was believed to be, by the free-hand use of a small (250 micra diameter at the tip) electrocautery. The wound was closed in layers and each animal was administered 1 ml of Dicrysticin Squibb intramuscularly. The animals were allowed to recover and particular care was taken that fluid and food intake was started as soon as possible after the operation, in order to prevent loss of weight.

Nine to eleven days after the operation the animals were anesthetized with Nembutal (45 mg/kg intraperitoneally) and perfused through the aorta with 0.9% NaCl solution followed by 10% formalin.

The chests, necks, and heads of the animals were then placed in 10% formalin until ready for histological study.

Two control animals were also studied. One was an animal in which the medulla was damaged but the dorsal nucleus was intact. In the other roughly one centimeter of the right cervical vagus had been removed at the level of the thyroid cartilage.

(2) Histological techniques. The medullae were removed from the skulls after approximately two months of fixation. Serial sections 30 micra thick were cut on a freezing microtome and stored in 10% formalin at a temperature of 5°C. Every sixth section was then stained with Kernechtrot, following the procedure outlined in Section B (3), to determine the extent of the lesion. If involvement of the dorsal nucleus was demonstrated, all the remaining sections were stained according to the procedure described by Nauta (1957), and studied for signs of degeneration, especially in the intramedullary vagal rootlets. The right cervical vagus nerves were also removed, embedded in 10% gelatin, cut longitudinally at a thickness of 30 micra and stained similarly. The cardiac branches of the vagus were carefully dissected out, embedded in paraffin, cut longitudinally and transversely at a thickness of 7 micra and stained according to the technique of Guillery, Shirra & Webster (1961).

D. Stimulation of the medulla.

(1) Physiological Experiments. 39 adult cats anesthetized with Chloralose (65 mg/kg intravenously) after ethyl chloride and ether induction were used. The surgical exposure of the floor of the fourth ventricle was carried out as described under Section B (2). In addition the right vagus nerve was exposed in the neck and freed for approximately 40 mm. The heads of the animals were fixed in a Kopf stereotaxic apparatus and so tilted downward as to allow insertion of a lucite platform carrying a pair of silver electrodes under the uncut cervical vagus, which was then freed of all connective tissue. The recording electrodes were connected to a Grass P-5 differential preamplifier.

The electrodes used to stimulate the medulla consisted of a pair of stainless steel insect pins (size 0, insulated to the tips with INSL-X 33, with a tip diameter varying from 20 to 40 micra), located approximately 300 micra apart; their D.C. resistance in saline was approximately 100 K Ω . They were connected to a Grass S4E stimulator through a Grass SIU-4 isolation unit. The stimulus used, except in special cases, was a square wave (amplitude 10 volts, duration .1 msec, frequency 20 per second, stimulation period 10 seconds). The effectiveness of the stimulus was tested at the beginning of each experiment by stimulating the hypoglossal nuclei, which lie quite superficially near the midline of the floor of the

fourth ventricle, a short distance rostral to the obex, and observing the effect of stimulation on the tongue. The electrode holder allowed minimal movements of the electrodes in steps of 100 micra in three orthogonal planes.

Arterial pressure and respiration from an esophageal balloon were monitored by two Statham pressure transducers and through two of the channels of an E.f.M. polygraph were fed through a beam splitter to one of the beams of the oscilloscope.

The electrical activity of the right vagus nerve was displayed on the other beam of the same oscilloscope, which was triggered by the stimulator. Still photographs of 200 superimposed sweeps as well as moving photographs of the nerve activity, arterial pressure and respiration were obtained. The oscilloscope, the recording and amplifying system and the audio monitoring system were previously described under Section A (1). A diagram of the experimental arrangement used is shown in Fig. 8.

(2) Histological localization of the sites of stimulation.

The same procedure outlined under section B (3) was used, but deposits of iron were produced at the tips of both electrodes.

(3) Analysis of compound action potentials. Measurements were made of latencies, following the stimulus,

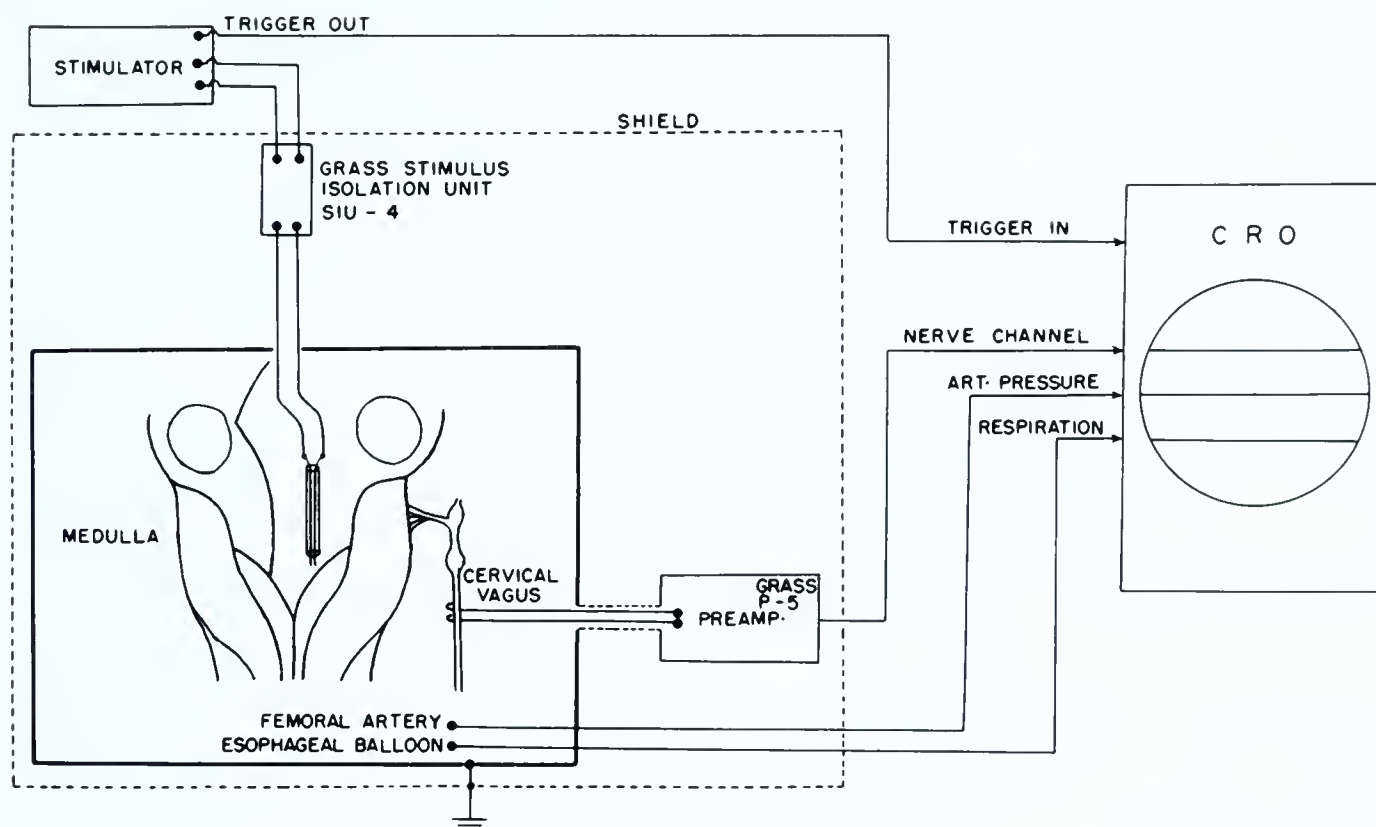


Fig. 8. Schematic diagram showing experimental arrangement used for the stimulation of the medulla.

of the component waves of the compound action potential in the vagus nerve and attempts were made to determine conduction velocities of fibers corresponding to the different waves.

The conduction distance was measured in the following way. An estimate of conduction distance between the vagal nuclei (dorsal nucleus and nucleus of the tractus) and the point of exit of the vagal rootlets from the medulla was obtained from cross sections of the medulla by measuring the distance between the nuclei and the point of exit of the rootlets, following the intra-medullary course of the vagal fibers. Measurements in vivo between the point of exit of the rootlets and the upper pole of the nodose ganglion were also obtained. In each animal at the end of the experiment the distance between the upper pole of the nodose ganglion and the midpoint between recording electrodes was measured. The approximate distance between vagal nuclei and upper pole of the nodose ganglion was found to be 28 mm. The total conduction distance ranged between 67 and 76 mm. Differences in conduction distance, due to the different positions of stimulation of the medulla, were a small proportion of the total conduction distance and therefore the conduction distance between vagal nuclei and point of exit of the rootlets was considered constant.

RESULTS

A. Recordings from vagal efferent fibers.

(1) "Single" fiber technique. Of the 28 cats used in this series only 14 gave reliable results. Of the various difficulties encountered in obtaining a suitable experimental preparation two should be mentioned. One was that, occasionally, without any obvious cause, the animals became unresponsive to phenyldiguanide and it was impossible to obtain reflex bradycardia by any other readily available means. The other was that at times it was not possible to keep the rectal temperature of the animals within physiological limits.

In these 14 animals recordings from 285 single fibers were obtained. The activity recorded was of five types. The term "random activity", used throughout the text, refers to activity uncorrelated to any of the observable body phenomena.

- a) Random activity, usually of small amplitude, uninfluenced by changes of heart rate (113 fibers).
- b) Activity of high amplitude synchronous either with inspiration or expiration, identified as activity from motor fibers to laryngeal muscles (160 fibers).

An example is shown in Fig. 9, record A.

- c) Activity of the baroreceptor type, eliminated by clamping of the ipsilateral common carotid artery (one fiber). The fiber exhibiting this type of activity is shown in Fig. 9, record B.

- d) Random activity of small amplitude which increased in frequency in time with slowing of the heart (7 fibers).

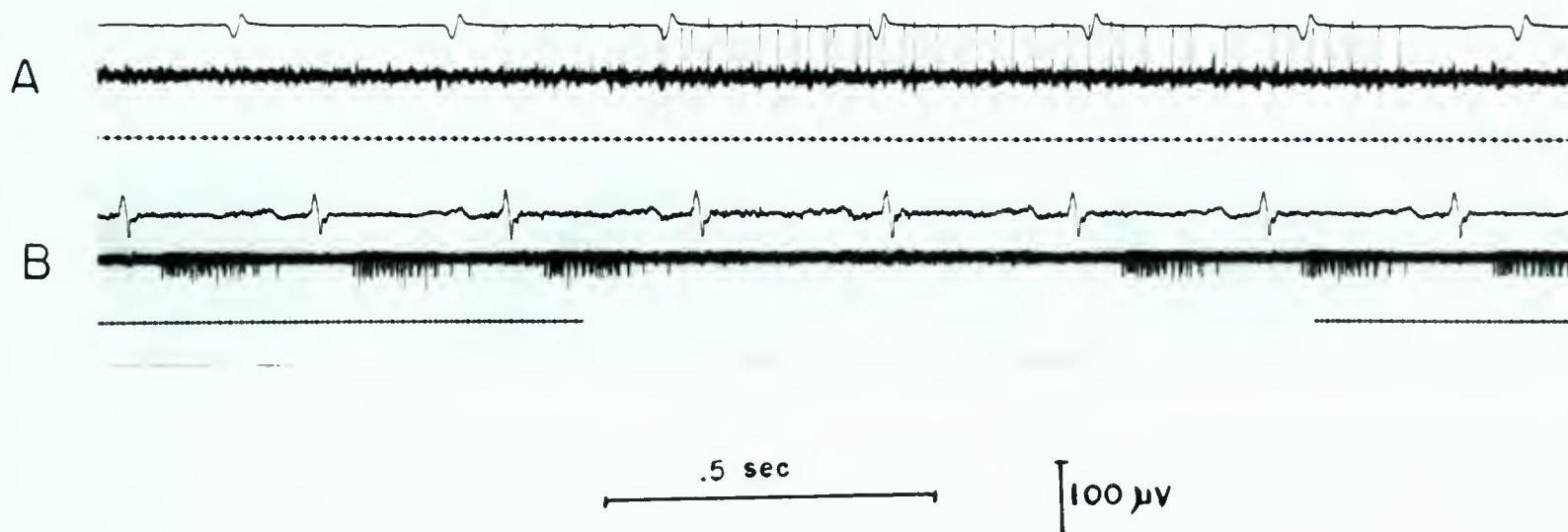


Fig. 9. Efferent activity in the cervical vagus.

In each record from above downwards :
electrocardiogram, electroneurogram and
event marker.

- A. Discharge of a motor fiber to a
laryngeal abductor muscle.
- B. Discharge of a baroreceptor fiber,
presumably from the carotid sinus area.
The ipsilateral carotid artery was
clamped below the bifurcation
approximately at the time of inter-
ruption of the event marker : the
interruption does not indicate the
exact times of clamping and unclamping
because the manoeuvres and the actuation
of the event marker switch were carried
out by two different operators.

Table V summarizes some physiological characteristics of these fibers. An example is shown in Figure 10 and a plot of frequency of discharge and heart rate for one of these fibers is shown in Fig. 11.

e) Random activity of high amplitude which decreased in frequency in time with bradycardia (4 fibers). An example is shown in Fig. 12.

(2) "Collision technique". Of the 10 cats in this series only 5 are included here. In approximately 30 attempts to produce changes in the compound action potential obtained from the electrically stimulated vagus nerve before, during and after phenyldiguanide induced slowing of the heart, it was not possible to detect any difference in the appearance of the compound action potential. Fig. 13 shows the results of a typical experiment in this series.

B. Recordings from the dorsal nucleus of the vagus

Of the 51 cats included in this series only 33 gave reliable results. Some of the animals not included died during or shortly after the exposure of the floor of the fourth ventricle or the decerebration, usually from loss of blood, or proved unsuitable because of loss of responsiveness to phenyldiguanide. 21 animals were under Chloralose anesthesia and twelve animals were artificially ventilated decerebrate spinal preparations. The animals in the latter group

TABLE V

PHYSIOLOGICAL CHARACTERISTICS OF

SOME EFFERENT FIBERS IN THE CERVICAL VAGUS

Fiber No.	Time of Onset of Increased Activity*	Duration of Bradycardia	Duration of Increased Activity	Maximum Frequency of Discharge
16-3	-1.2 sec	23 sec	20 sec	52/sec
26-4	-0.4 sec	> 30 sec	30 sec	45/sec
26-8	-1.5 sec	24 sec	15 sec	49/sec
27-1	-1.2 sec	26 sec	20 sec	19/sec
27-3	-1.5 sec	28 sec	18 sec	22/sec
28-4	-1.2 sec	35 sec	22 sec	12/sec
26-10	+2.3 sec	21 sec	5 sec	30/sec

*Times indicated are relative to time of onset of bradycardia: negative numbers indicate that the increased activity preceded in bradycardia, positive numbers that the bradycardia preceded the increased activity.

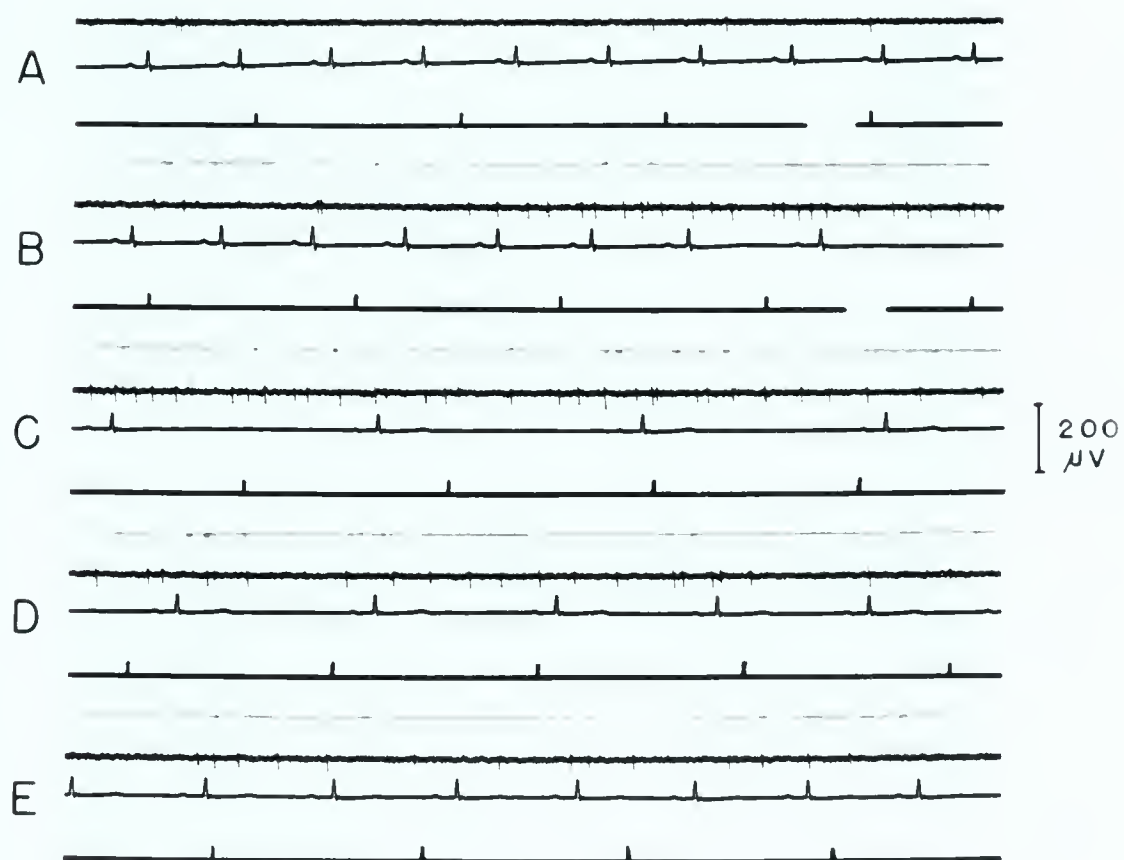


Fig. 10. Cervical vagus unit 28-4. Continuous records.

In each from above downwards : electro-neurogram, electrocardiogram and event marker (vertical bars one second apart). Between the two interruptions on the event marker phenyldiguanide (100 μ g intravenously) was administered : bradycardia and increased frequency of discharge of the fiber are seen.

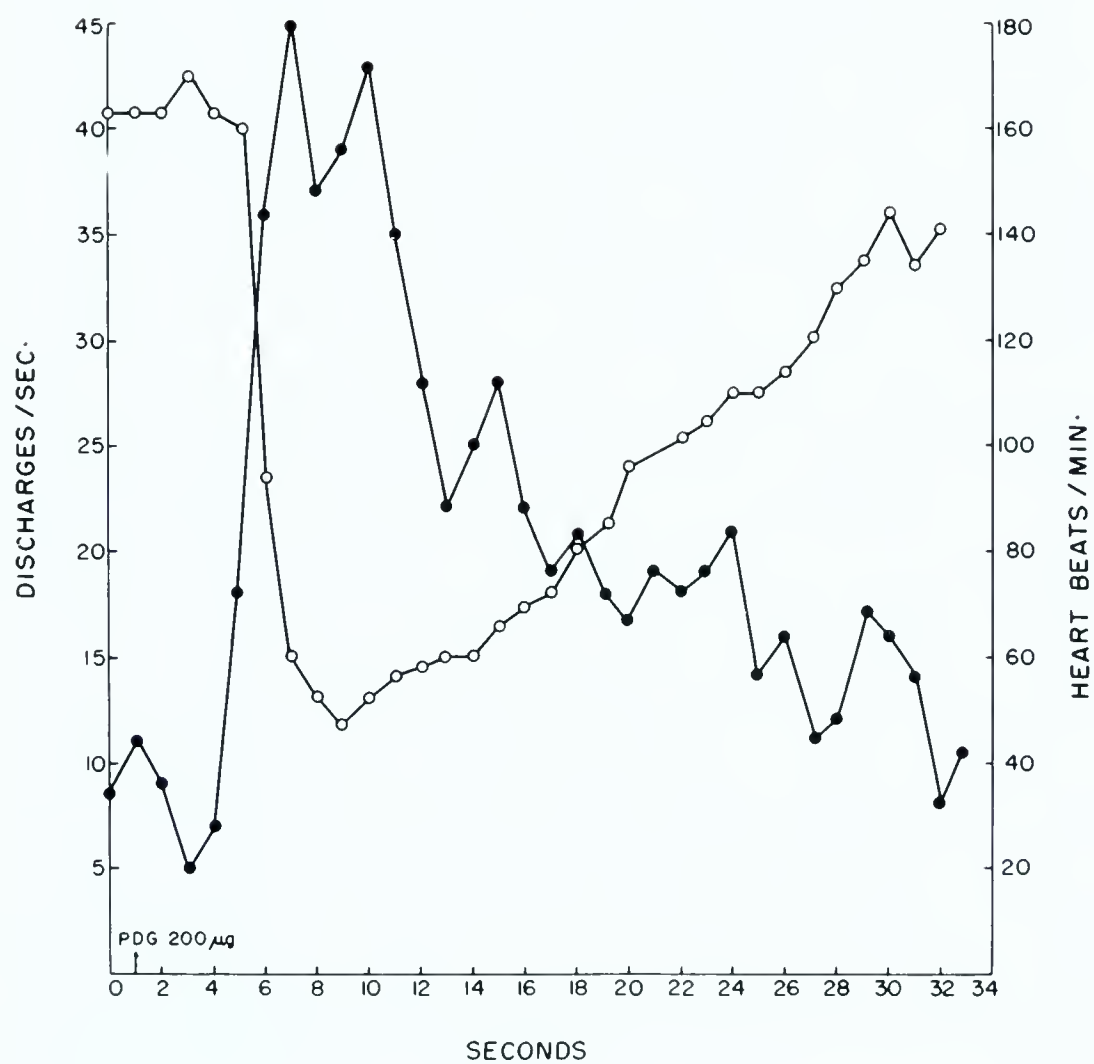


Fig. 11. Cervical vagus unit 26-4. Plot of heart rate and frequency of discharge versus time over consecutive one-second intervals.

○ Heart rate.

● Frequency of discharge.

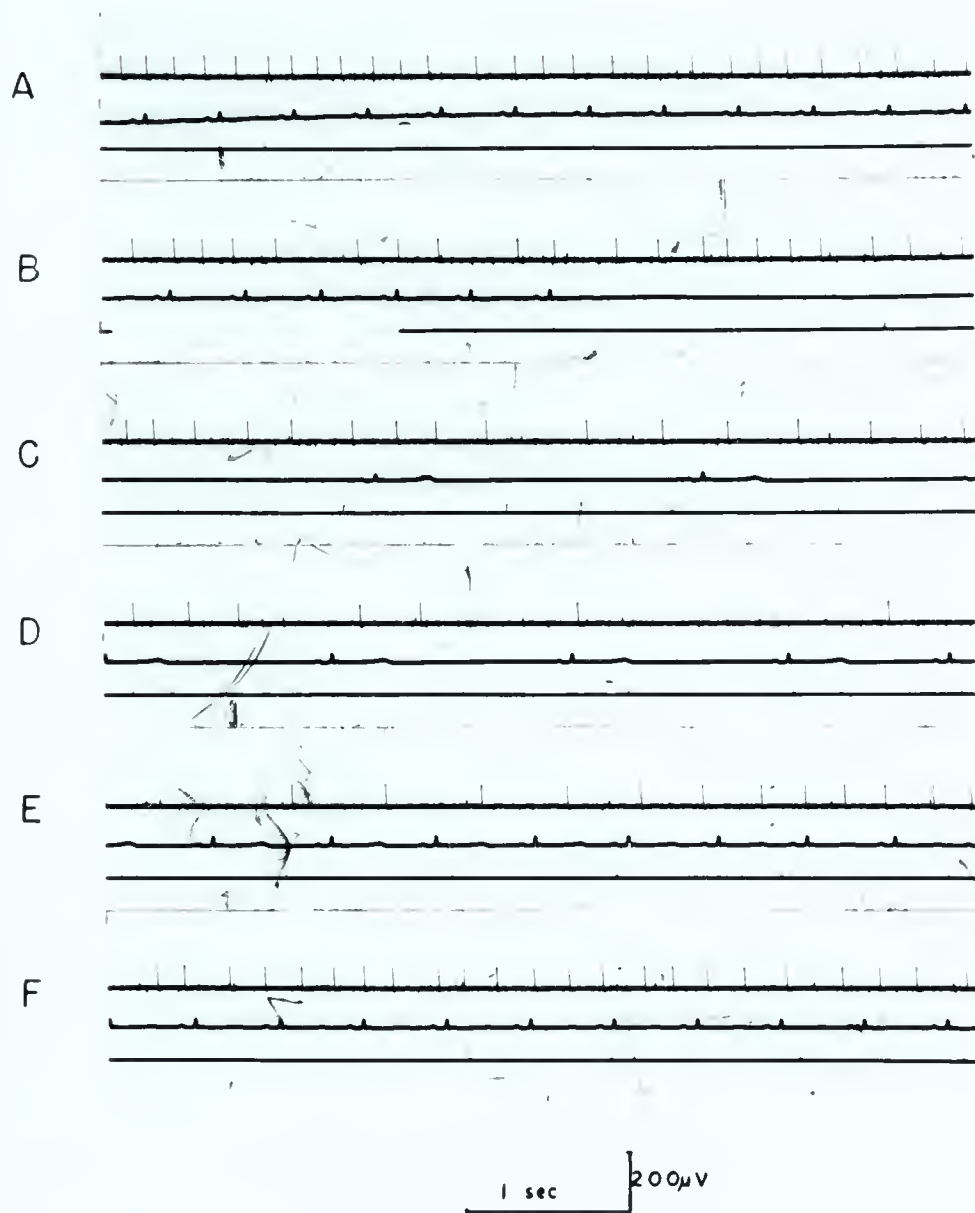


Fig. 12. Cervical vagus unit 27-3. Continuous records. In each from above downwards : electroneurogram, electrocardiogram and event marker. During interruption of event marker phenyldiguanide ($100 \mu\text{g}$ intravenously) was administered : bradycardia and decreased frequency of discharge are seen.

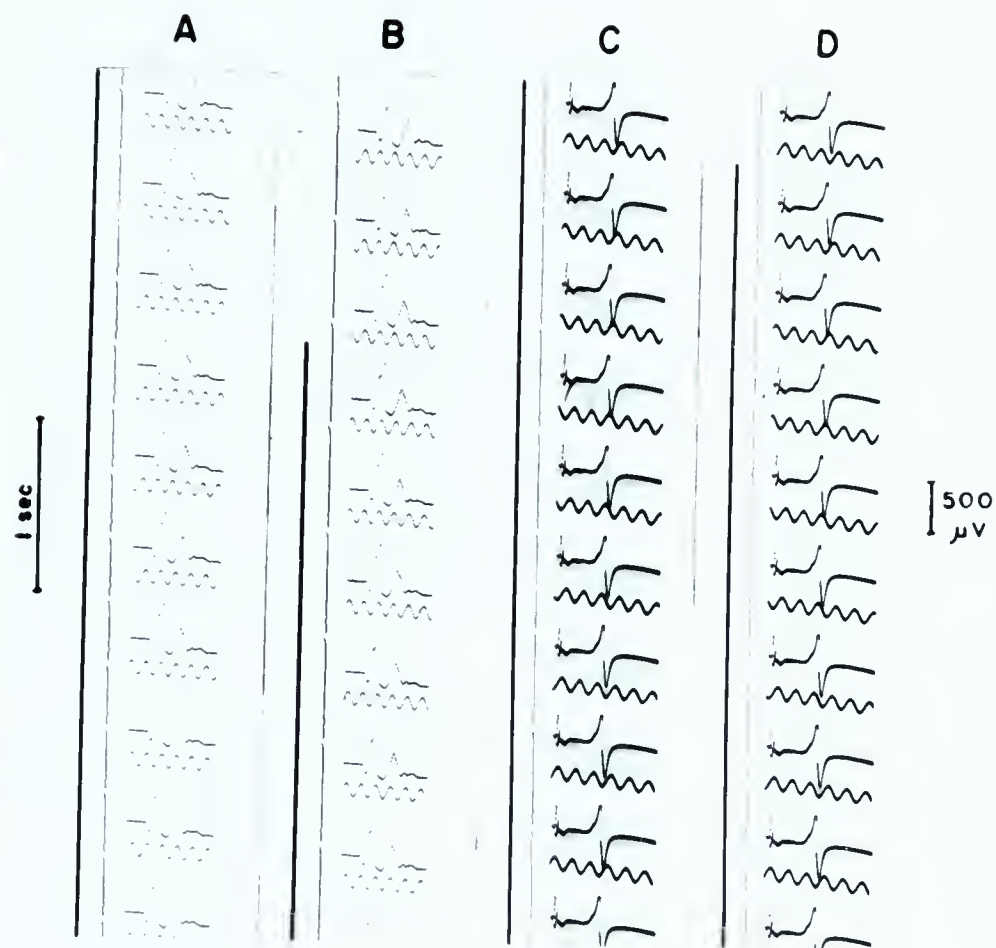


Fig. 13. Cat no.22. Compound action potentials of the cervical vagus before and after administration of phenyldiguanide (100 μ g intravenously). In each record : event marker and electrocardiogram on the vertical axis, electroneurogram and time calibration on the horizontal axis.

A. Control A and B fibers action potentials.
Time mark 1 msec.

B. Same as A, after phenyldiguanide.

C. Control C fibers action potential.
Time mark 10 msec.

D. Same as C, after phenyldiguanide.

were used for two reasons: to see whether the activity of dorsal nucleus units in decerebrate animals was in any way different from the activity found in animals anesthetized with Chloralose, and to eliminate by spinal section some of the recording artefacts present in animals breathing spontaneously. The results are presented together because no difference was found between the activity recorded in the two groups of animals.

Of 252 penetrations aimed at the dorsal nucleus 50 were successful as judged by histological study. Of these, 28 yielded no activity before, during or after bradycardia, 6 showed a markedly increased activity following PDG administration and in time with bradycardia, one showed increased activity in time with bradycardia following carotid sinus stimulation and one showed decreased activity in time with bradycardia following PDG administration. A summary of the types of activity recorded and the location of the successful penetrations is shown in Fig. 14. An example of a unit showing an increased frequency of discharge in time with bradycardia is shown in Fig. 15 and a summary of some physiological characteristics of the units exhibiting a similar behavior is shown in Table VI. A plot of frequency of discharge and heart rate for one of these units is presented in Fig. 16. Finally Fig. 17 shows a cross section of the medulla in which an iron deposit

LOCATION OF 50 PENETRATIONS IN THE DORSAL
NUCLEUS OF THE VAGUS

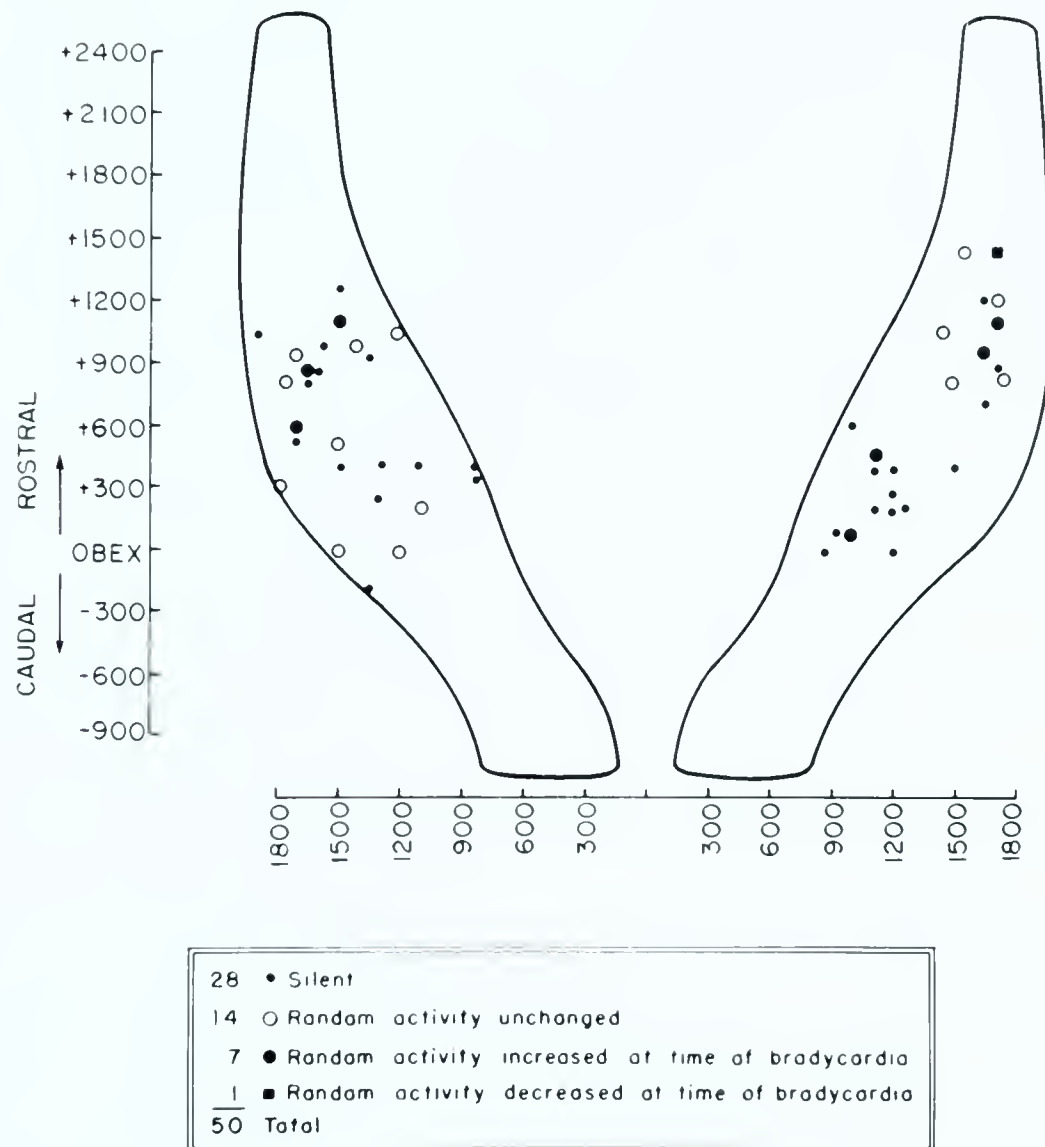


Fig. 14. Location and type of electrical activity of 50 single units in the dorsal nucleus of the vagus.
Distances in μ .

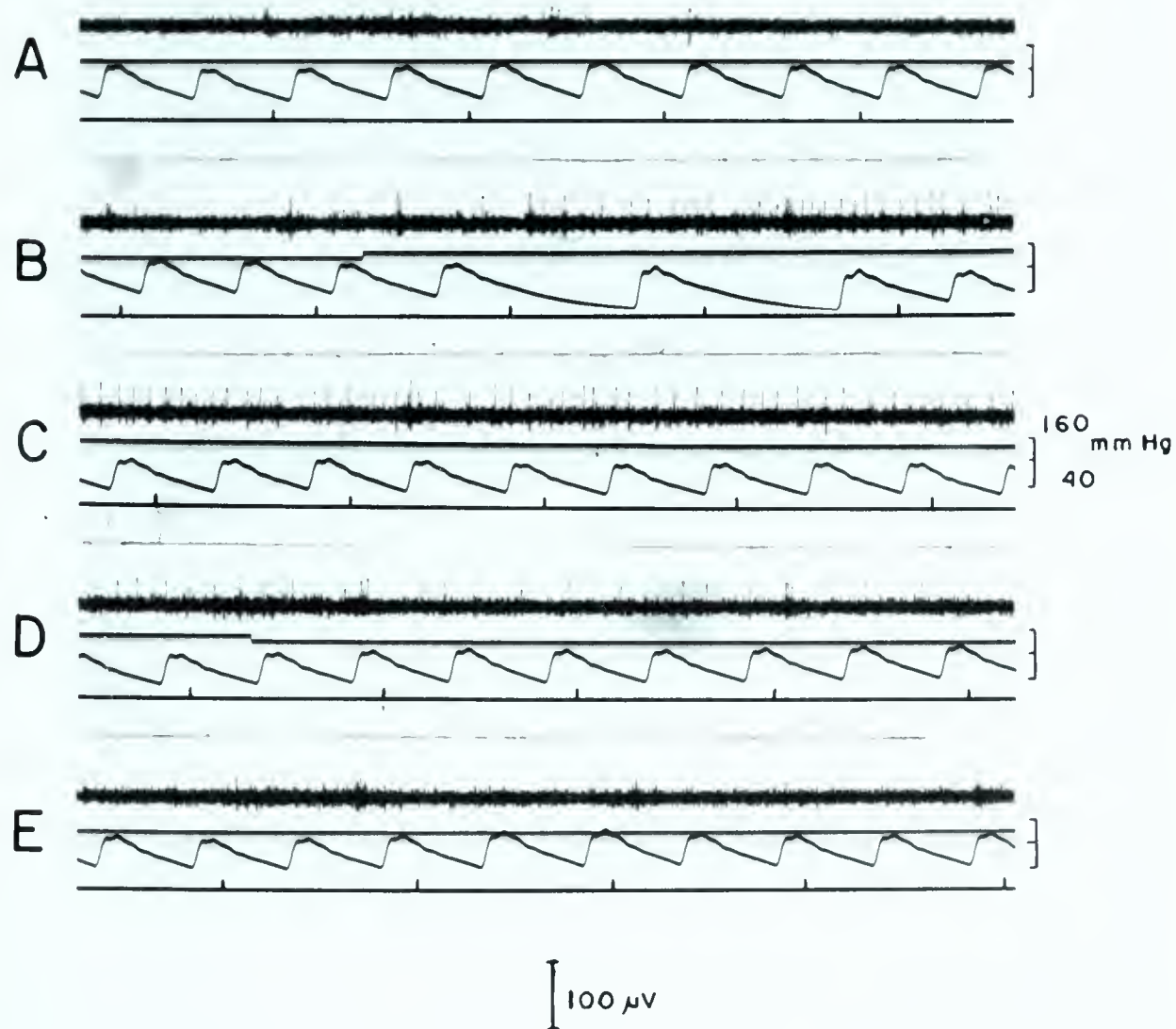


Fig. 15. Dorsal nucleus unit 29-7. Continuous records.
 In each from above downwards : electro-neurogram, event marker, arterial pressure and time markings (vertical bars 1 second apart).
 Between the two steps on the event marker the ipsilateral carotid sinus was stimulated: bradycardia and increased frequency of discharge of the unit are seen.

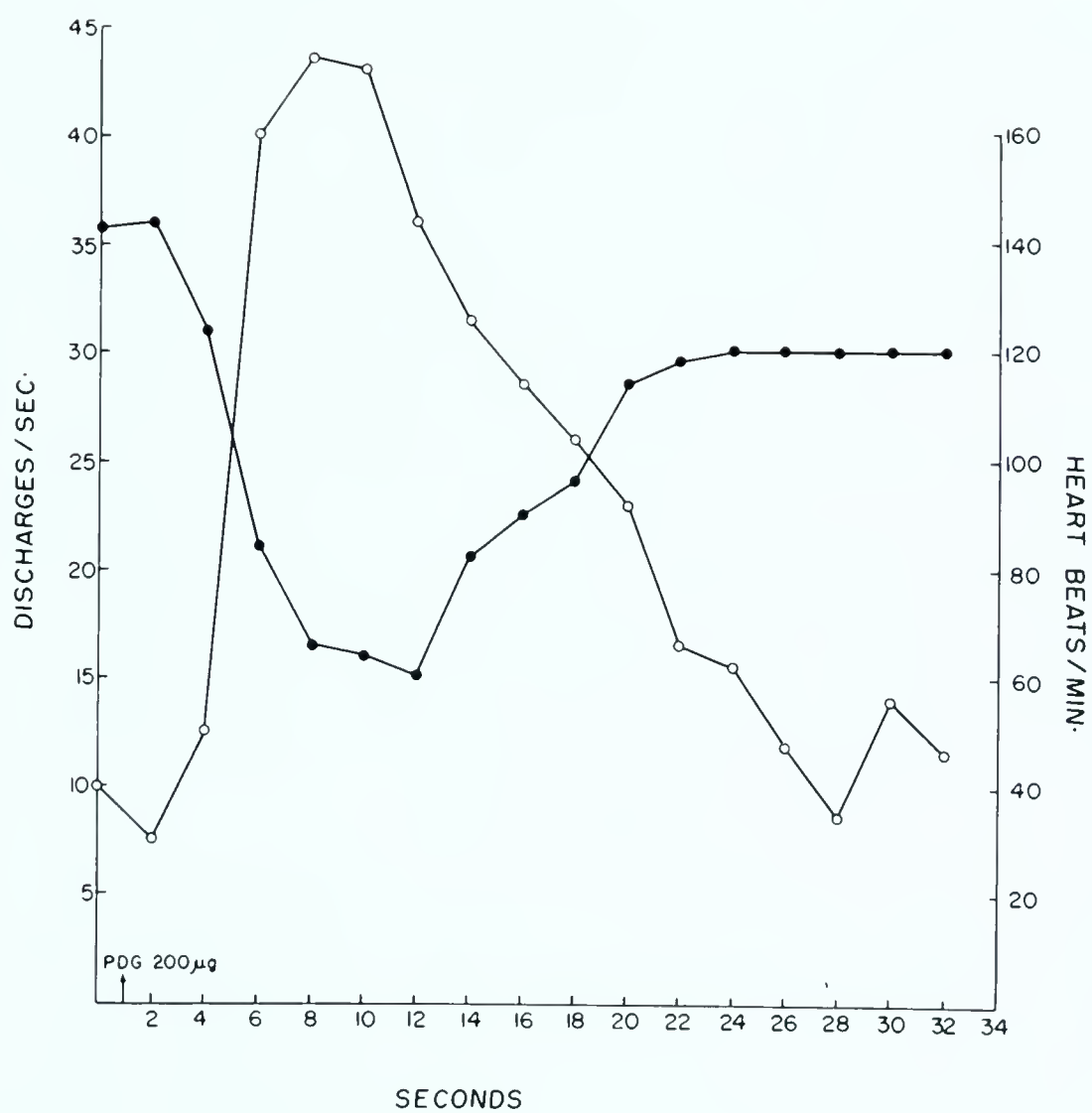


Fig. 16. Dorsal nucleus unit 32-9. Plot of heart rate and frequency of discharge versus time over consecutive intervals of 2 seconds.

o Frequency of discharge.

● Heart rate.

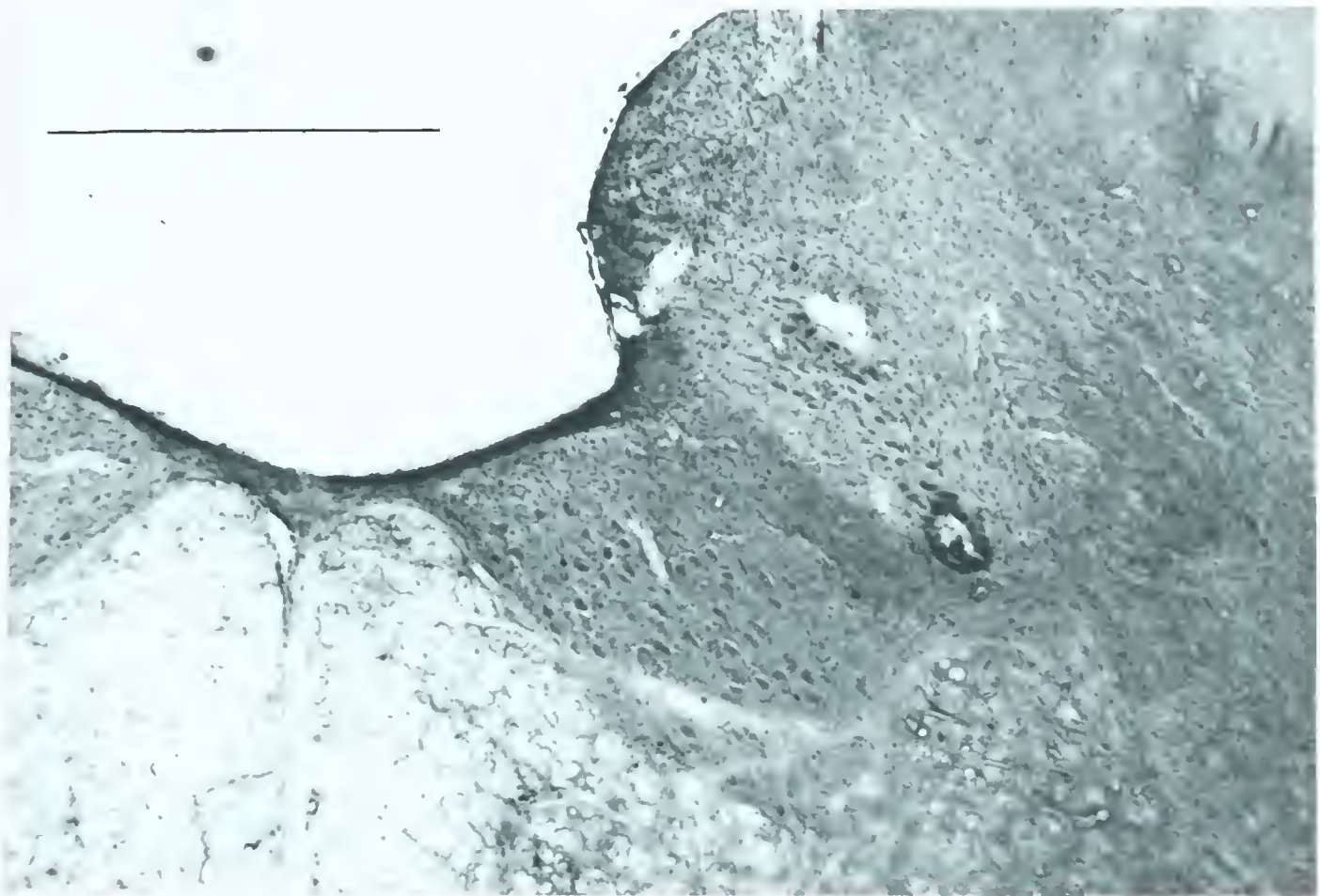


Fig. 17. Cat 28-D. Iron deposit within the dorsal nucleus of the vagus in a cross section of the medulla.
Kernechtrot and potassium ferrocyanide stain.
Calibration 1 mm.

TABLE VI

PHYSIOLOGICAL CHARACTERISTICS OF SOME
UNITS IN THE DORSAL NUCLEUS OF THE VAGUS

Unit No.	Time of Onset of Activity*	Duration of Bradycardia	Duration of Increased Activity	Maximum Frequency of Discharge
19-3	+1.1 sec	11 sec	7 sec	34
32-5	+1. sec	39 sec	16 sec	17
32-7	+2.4 sec	15 sec	6 sec	26
32-9	+1 sec	8 sec	21 sec	44
48-9	+1.3 sec	16 sec	14 sec	30
46-2	-0.5 sec	16 sec	13 sec	42
29-7**	+0.3 sec	14 sec	13 sec	17

*Times indicated are relative to time of onset of bradycardia; negative numbers indicate that the increased activity preceded the bradycardia, positive numbers that the bradycardia preceded the increased activity.

**Unit 29-7 Bradycardia followed carotid sinus stimulation.

within the dorsal nucleus of the vagus is visible.

During this study recordings of activity from medullary structures other than the dorsal nucleus were obtained. The histological identification of these locations was obtained by the usual method. Activity was recorded from the reticular formation, the hypoglossal nucleus, the nucleus ambiguus, the nucleus intercalatus and the nucleus of the tractus solitarius. Activity from units in the reticular formation and the hypoglossal nucleus showed no changes related to slowing of the heart. Activity from two units exhibiting a respiratory rhythm was recorded from the nucleus ambiguus (see Fig. 7, record C). Random activity was recorded from six units in the nucleus intercalatus; five of these showed no change and one showed a decrease in activity during bradycardia. Finally activity from seven units was recorded from the nucleus of the tractus solitarius: one unit exhibited a cardiac rhythm, two a respiratory rhythm and four a random rhythm, but none of them showed either an increase or a decrease of activity during bradycardia, except for the changes in the frequency of the trains of impulses accompanying slowing of the heart and respiration in the units with a cardiac or respiratory rhythm.

In an attempt to obtain an estimate of the volume of tissue from which activity could be recorded

the activity of a single unit in the dorsal nucleus was studied. When a position of the tip of the electrode was found at which maximum amplitude could be recorded, the same activity, clearly visible above the noise level but diminished in amplitude, could be recorded at 150 micra dorsal to the position of maximum amplitude and 100 micra ventral to it. Taking the smaller of the two figures as an approximate estimate of the distance from which activity from firing units could be recorded, it was concluded that the tip of the electrodes could record from units contained in a sphere of nervous tissue approximately 200 micra in diameter.

C. Lesions of the dorsal nucleus of the vagus.

Of the 17 animals used in this series lesions involving the dorsal nucleus were demonstrated histologically only in 5. The dorso-ventral and rostro-caudal extents of the lesions, as determined by the study of serial sections of the medulla, are outlined in Fig. 18. The lateral extent of the lesions is not outlined because the whole lateral extent of the nucleus was involved. The appearance of the lesion at four different levels in cross sections of the medulla of one animal is shown in Fig. 19.

The criterion used in deciding that degeneration was present was the observation of axonal debris for a length of the nerve fiber. In some cases

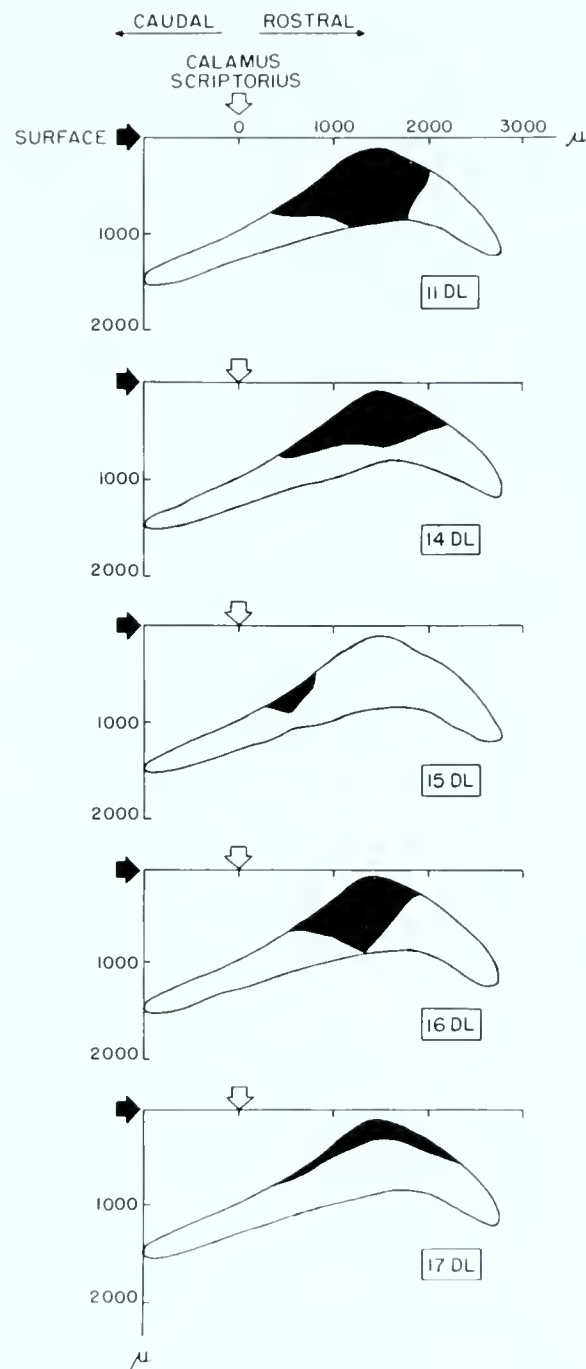


Fig. 18. Outlines of dorso-ventral and rostro-caudal extent of lesions of the dorsal nucleus of the vagus in five animals. The dorsal nucleus is outlined in each diagram and the black areas indicate the size of the lesion. Distances in μ .

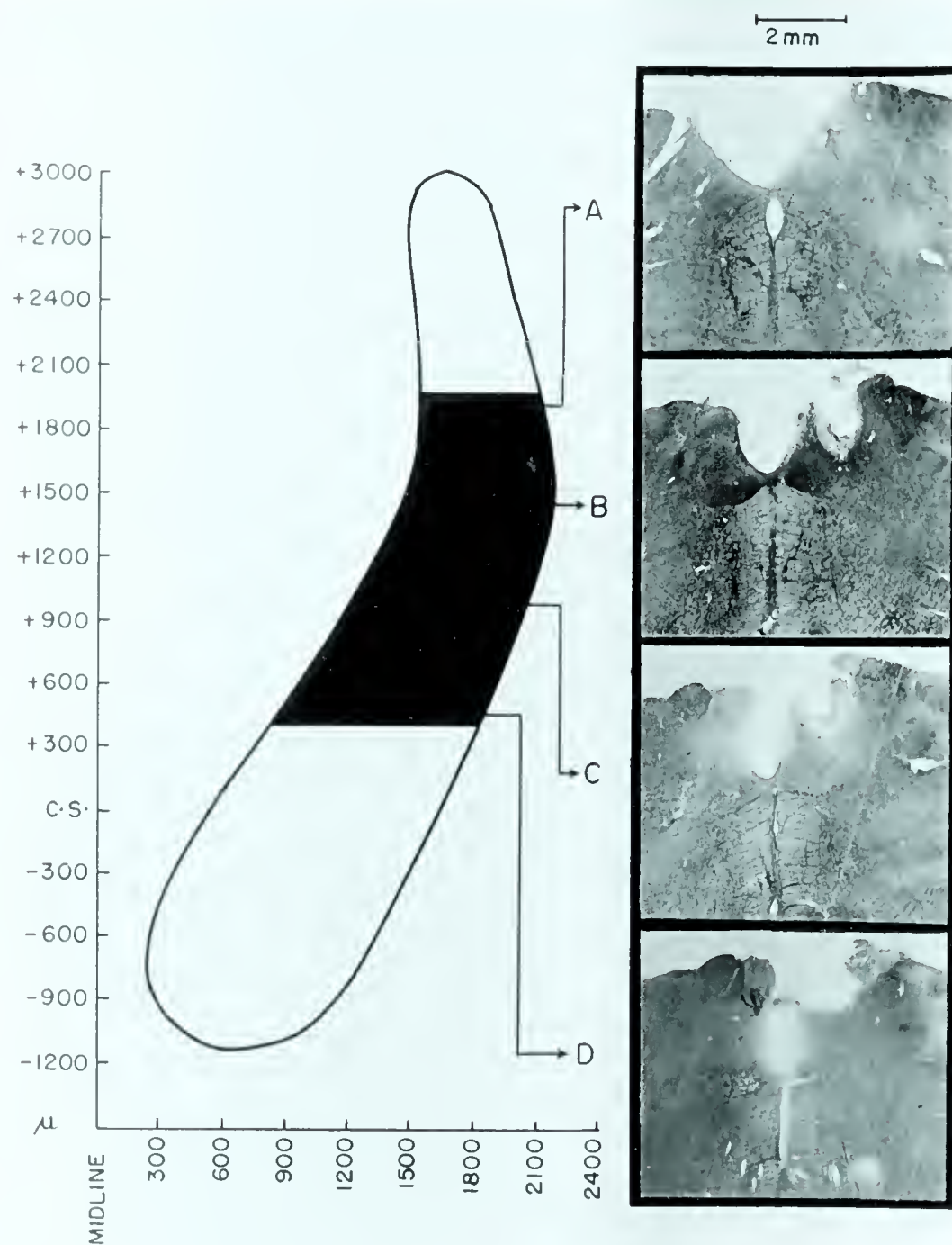


Fig. 19. Cat 11-DL. Lesion in the dorsal nucleus. On the left outline of the nucleus : the black area indicates the lesion. On the right cross sections of the medulla at four different levels showing size and location of lesion. 30 μ sections. Nauta stain.

the degenerating axon could be seen for a fairly long length of fiber but in most cases it could only be seen for a short segment.

In all five animals axonal degeneration could be clearly seen in the intramedullary rootlets of the vagus as well as in the cervical vagus. Figures 20 and 21 show typical examples of degenerating axons in the intramedullary rootlets and in the cervical vagus. For comparison, Fig. 22 shows widespread axonal degeneration in the cervical vagus of one control animal in which a one centimeter portion of the cervical vagus had been removed and the animal allowed to survive for ten days. In addition, because in some of the animals with lesions of the dorsal nucleus there was also a lesion of the nucleus of the tractus solitarius, one animal (3-DL), in which the nucleus of the tractus was damaged and the dorsal nucleus was intact, was studied in order to exclude the possibility that degeneration in the peripheral vagus was due to the damage of the nucleus of the tractus. The appearance of the lesion in this animal is shown in Fig. 23. No degeneration could be observed in the intramedullary rootlets and in the cervical vagus of this animal. Attempts were made to trace the degenerating axons from the damaged nucleus of the tractus, and some were found ending in the dorsal nucleus of the vagus. The significance of this finding will be discussed later.



Fig. 20. Cat 15-DL. Degeneration in an intramedullary vagal rootlet. Axonal debris from degenerated fibers, as well as normal fibers are seen.
25 μ section. Nauta stain. Calibration 10 μ .

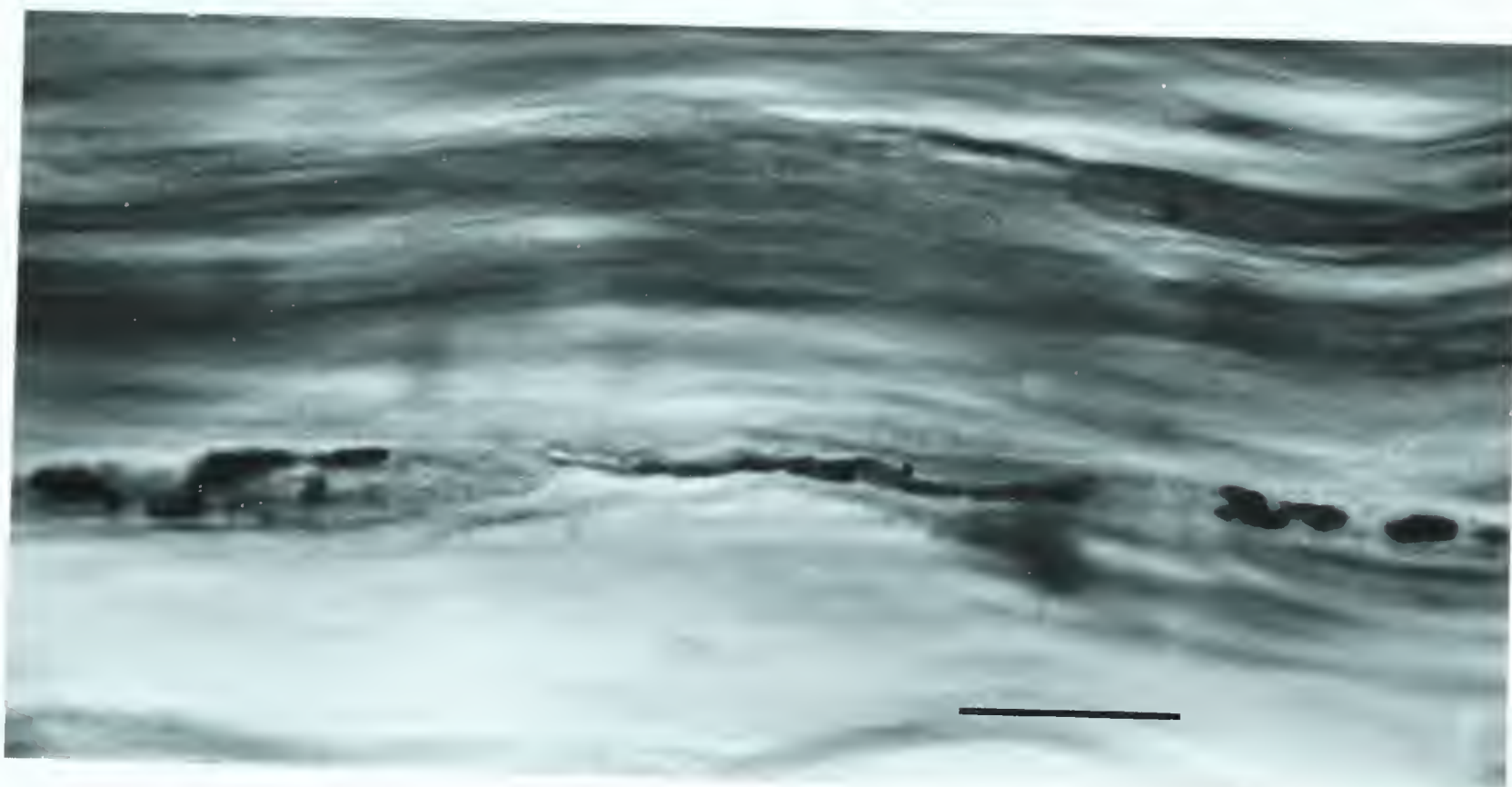


Fig. 21. Cat 11-DL. Degeneration in the cervical vagus. A completely degenerated fiber as well as normal fibers are seen. 25 μ section. Nauta stain. Calibration 10 μ .



Fig. 22. Cat 1-PV. Diffuse axonal degeneration in the cervical vagus following infranodose vagotomy.
25 μ section. Nauta stain. Calibration 50 μ .

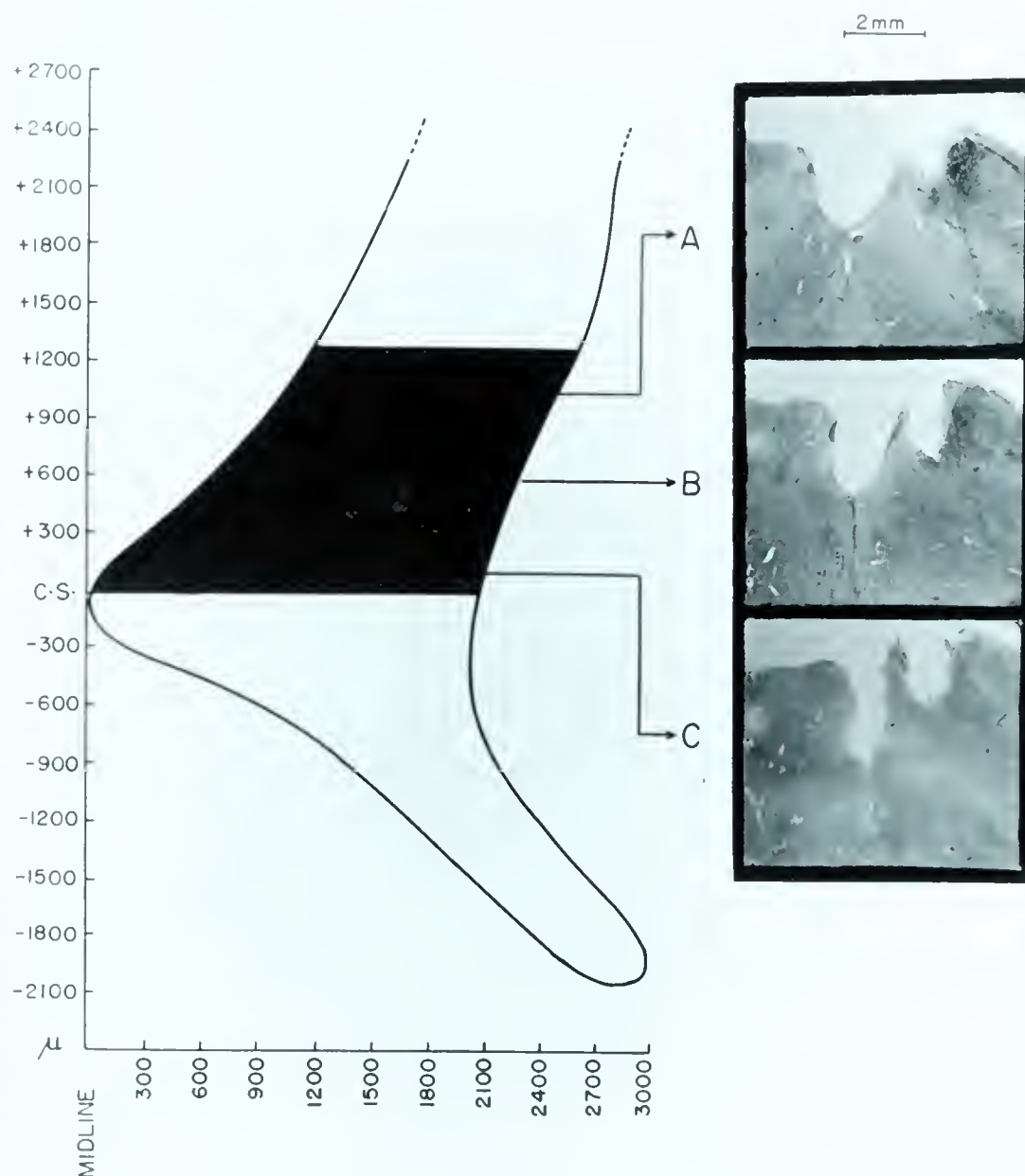


Fig. 23. Cat 3-DL. Lesion in the nucleus of the tractus solitarius. On the left outline of the tractus and its nucleus : the black area indicates the lesion. On the right cross sections of the medulla at three different levels showing size and location of lesion. 30 μ sections. Nauta stain.

With regard to the cardiac branches, degeneration was seen in only two of the five animals. Fig. 24 shows degeneration in a cardiac branch. Fig. 25 summarizes the results of this group of experiments, including a semi-quantitative assessment of the amount of degeneration present.

D. Stimulation of the medulla.

The results from this series of 39 cats will be presented under two headings: systematic exploration and stimulation of points producing bradycardia.

(1) Systematic exploration. Of 9 cats used in this series a complete exploration could be carried out only in 5. After the stimulating electrodes had been positioned at the obex with the aid of a binocular dissecting microscope, points 500 micra apart, on the surface and at a depth of 1 mm, were stimulated. Compound action potentials in the vagus nerve were recorded during stimulation of several locations on the surface and a depth of 1 mm. Physiological responses were also observed and recorded. The results of a typical exploratory experiment are shown in Figures 26 and 27. Composite pictures of the physiological responses obtained in the five animals are shown in Figures 28 and 29.

(2) Stimulation of selected points. In this group of 30 cats results were obtained in 20. In these

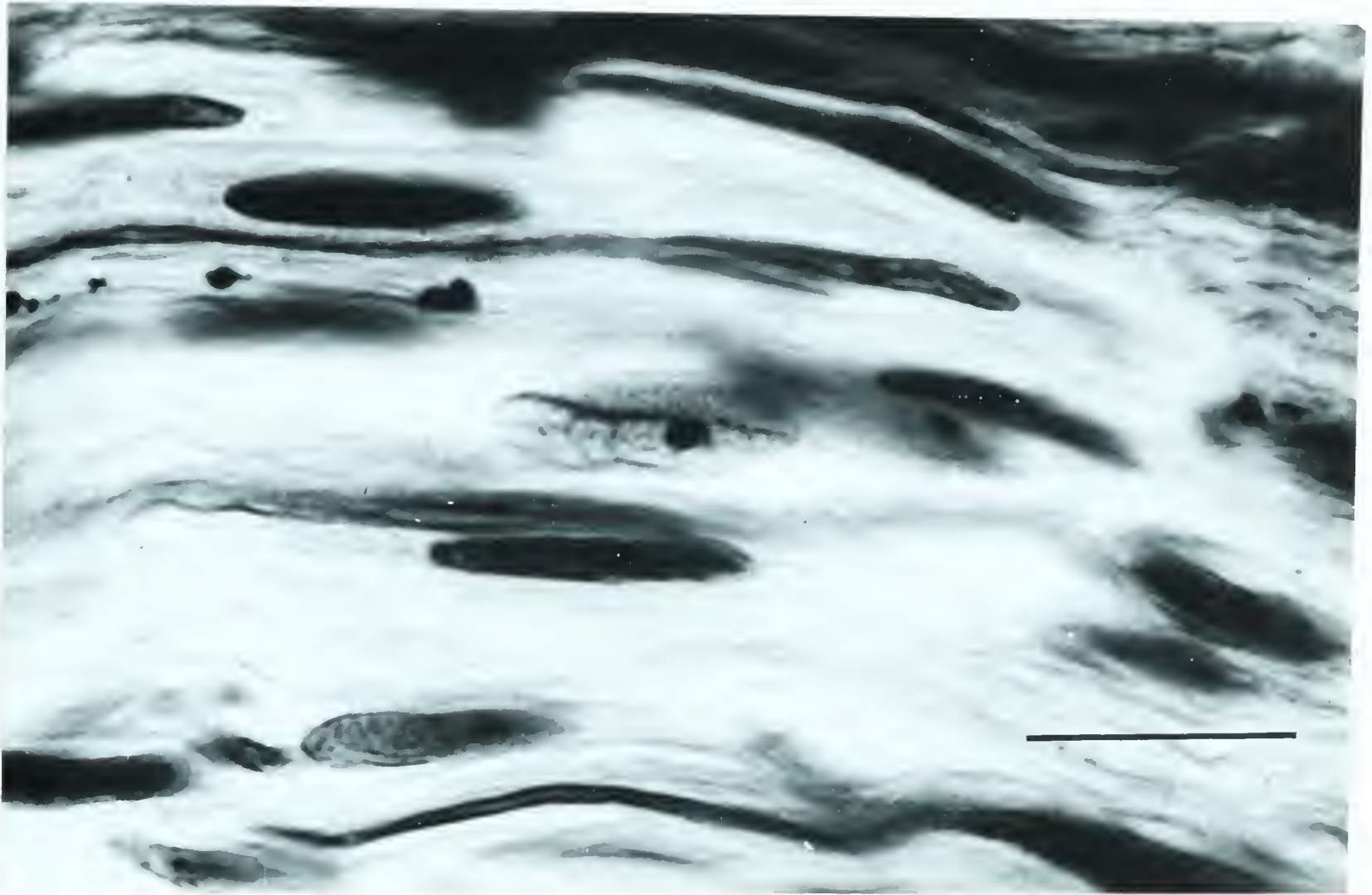


Fig. 24. Cat 16-DL. Degeneration in a cardiac branch.
Axonal debris from a degenerated fiber is
seen.
7 μ section. Guillery et al. stain.
Calibration 10 μ .

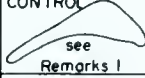
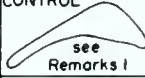





CAT #	EXTENT of LESION	INTRAMEDUL- LARY ROOTLETS	CERVICAL VAGUS	CARDIAC BRANCHES	REMARKS
1 - PV	CONTROL 	see Remarks 2	+++++	+++++	① A ONE CM SEGMENT OF THE CERVICAL VAGUS REMOVED ② MEDULLA NOT STUDIED
3 - DL	CONTROL 	—	—	see Remarks2	① LESION IN THE NUCLEUS OF T-SOLITARIUS ② CARDIAC BRANCHES NOT STUDIED
11 - DL		+++	++	see Remarks	NO SATISFACTORY PREPARATIONS OF CARDIAC BRANCHES OBTAINED
14 - DL		+++	++	< +	
15 - DL		+++	++	—	
16 - DL		++	++	+	
17 - DL		++	+	—	

Fig. 25. Diagram showing size of lesions in the dorsal nucleus of the vagus and amount of degeneration present in the intra-medullary rootlets, cervical vagus and cardiac branches.
+++++ amount of degeneration present in a vagotomized animal.
— no degeneration.

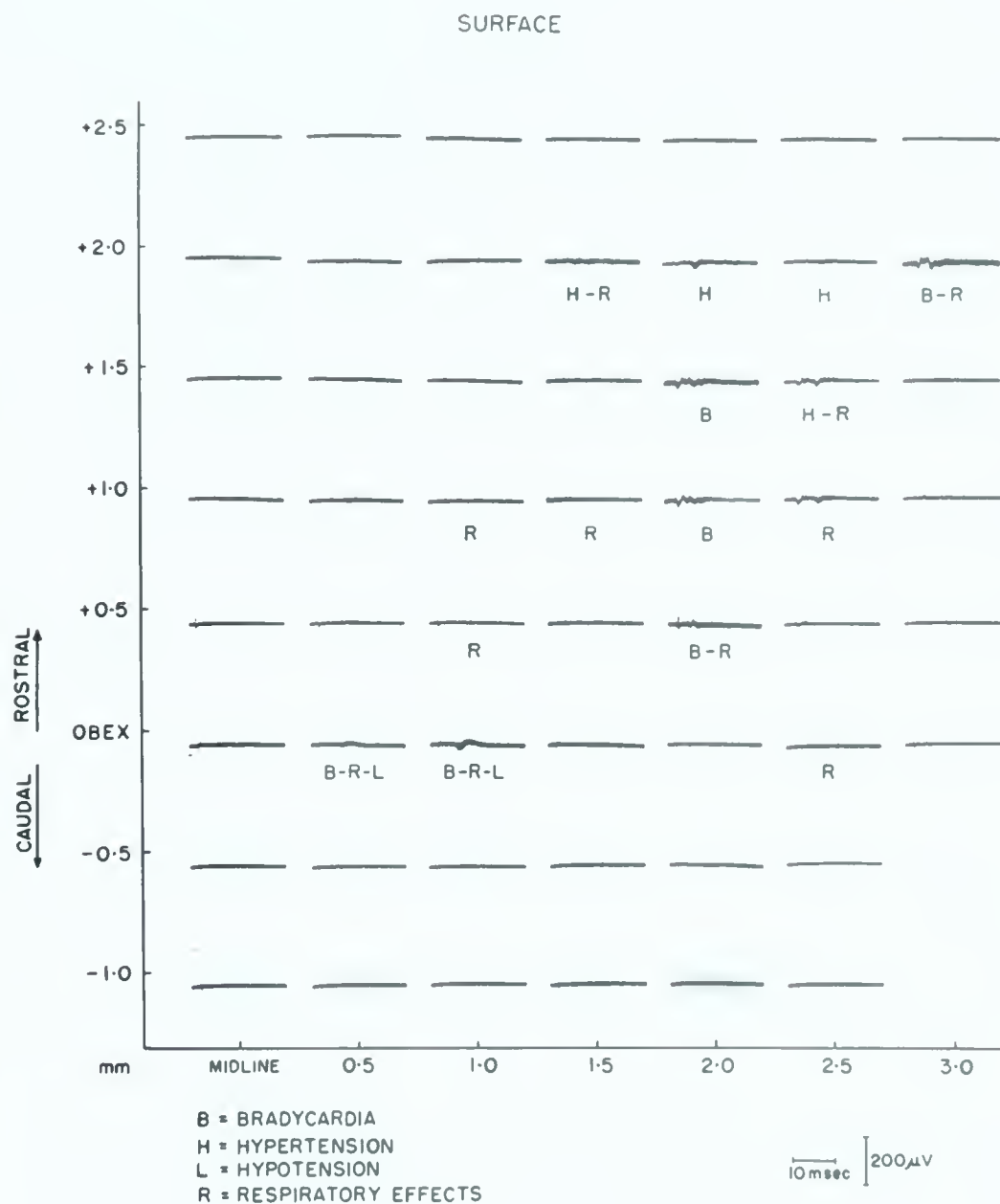


Fig. 26. Cat 30-DV. Compound action potentials recorded from the right cervical vagus during stimulation of points 0.5 mm apart on the surface of the right side of the floor of the IVth ventricle. Physiological responses are indicated below each record, which is composed of approximately 200 superimposed sweeps. Distances in mm.

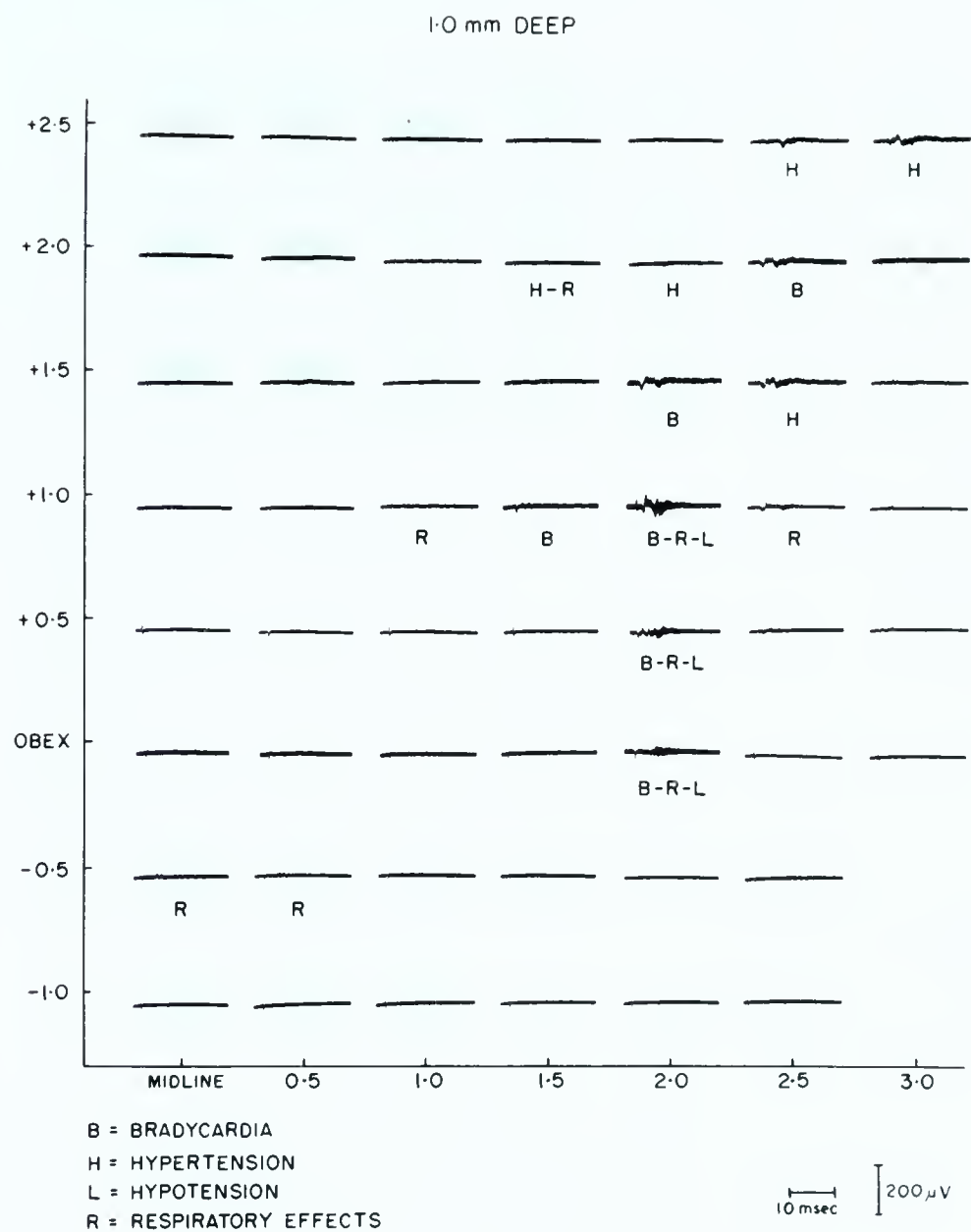


Fig. 27. Cat 30-DV. Compound action potentials recorded from the right cervical vagus during stimulation of points 0.5 mm apart, 1 mm below the surface of the right side of the floor of the IVth ventricle. Physiological responses are indicated below each record, which is composed of approximately 200 superimposed sweeps. Distances in mm.

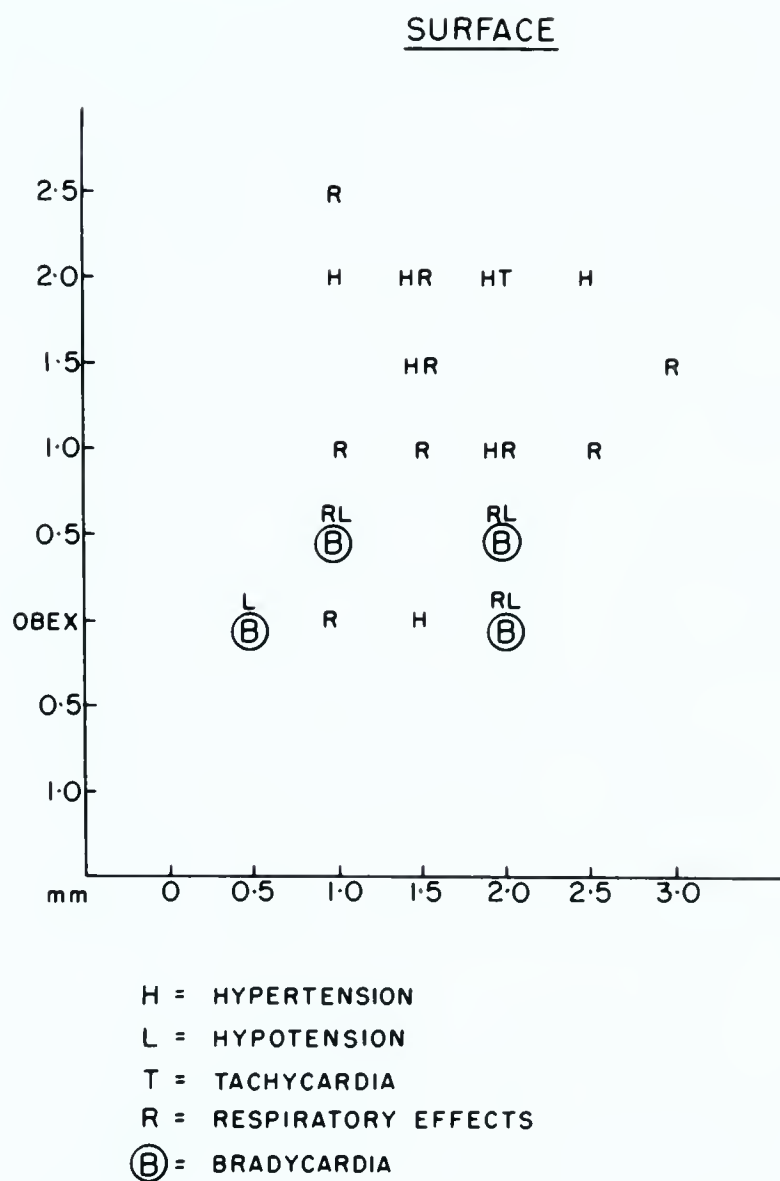


Fig. 28. Summary of physiological responses in five animals during electrical stimulation of points 0.5 mm apart on the surface of the floor of the IVth ventricle. Distances in mm.

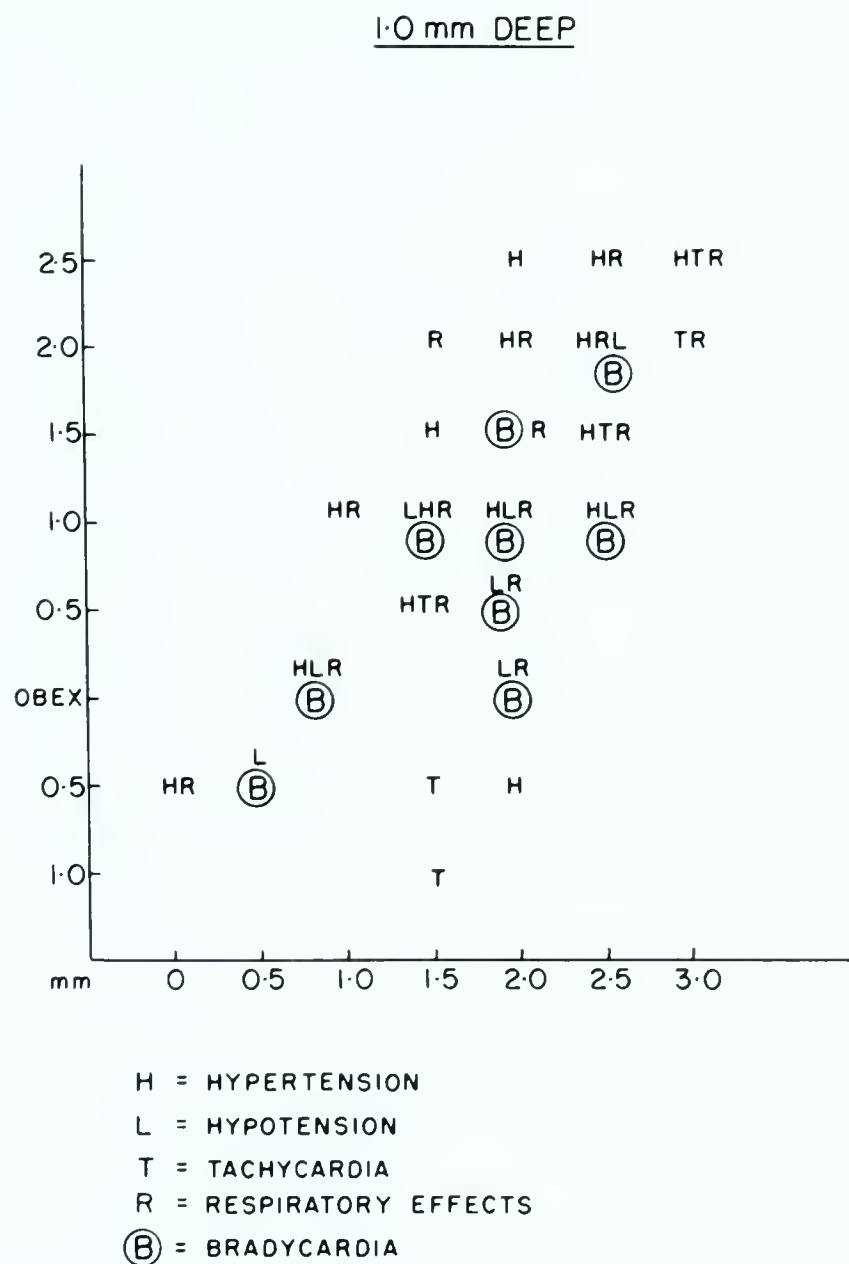


Fig. 29. Summary of physiological responses in five animals during electrical stimulation of points 0.5 mm apart, 1 mm below the surface of the right side of the floor of the IVth ventricle.
Distances in mm.

animals selected points of the medulla were stimulated, with two objectives in mind: to identify histologically locations which on electrical stimulation produced slowing of the heart and to stimulate locations which were thought to be within the dorsal nucleus of the vagus. At the same time physiological responses and compound action potentials in the vagus nerve following stimulation were recorded. The locations of stimulation were later determined histologically and correlated with the physiological effects and the electrical responses in the ipsilateral vagus.

Stimulation of 22 locations produced bradycardia: the iron deposits in these positions were found within the nucleus or the tractus solitarius (Fig. 30). On the other hand, 10 positions, which on electrical stimulation failed to produce slowing of the heart or any other changes which could be observed with the experimental arrangement used, were located within the dorsal nucleus of the vagus (Fig. 31). An example of a pair of iron deposits within the dorsal nucleus is shown in Fig. 32. In the animals in which the 10 stimulations of the dorsal nucleus failed to produce bradycardia, slowing of the heart could be produced by stimulation of the tractus solitarius or its nucleus. A noteworthy finding was that stimulation of medullary structures outside the nucleus of the tractus never produced bradycardia.

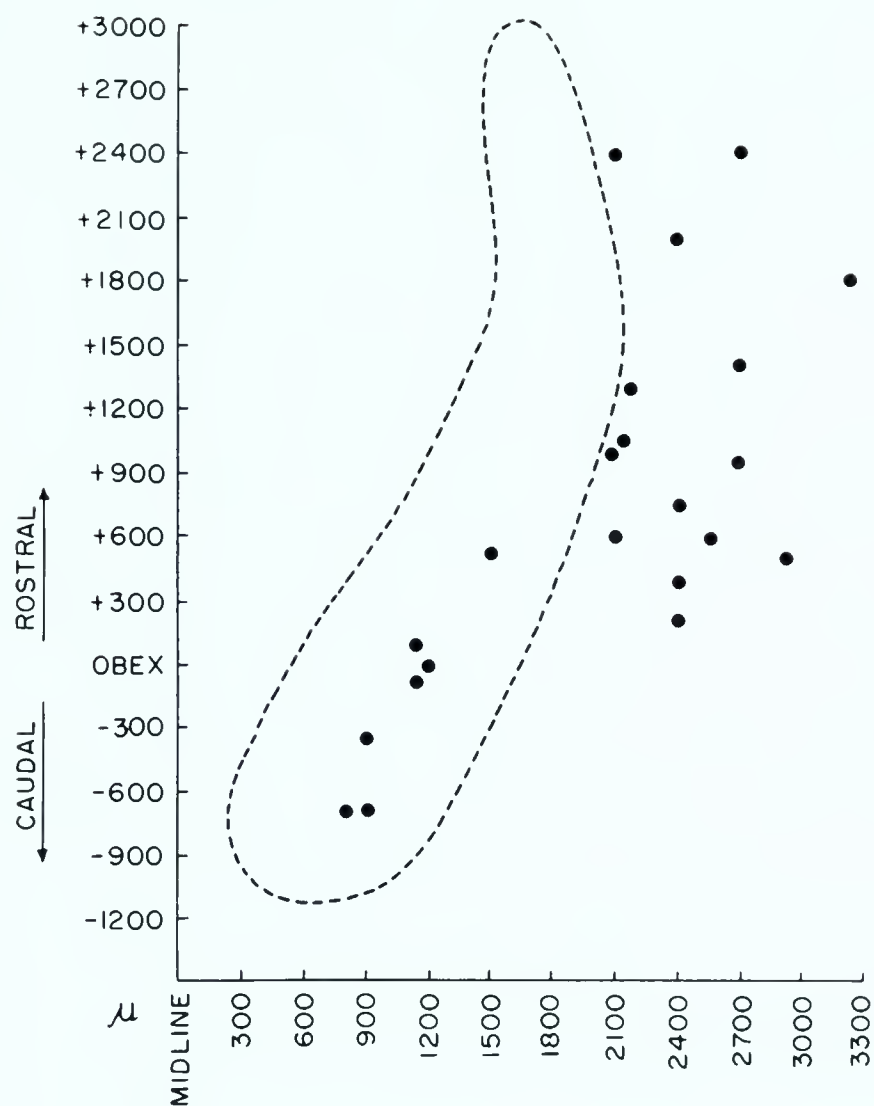


Fig. 30. Location of 22 iron deposits in sites which produced bradycardia on electrical stimulation. The dashed outline indicates the surface projected extent of the dorsal nucleus. All the iron deposits were found within the tractus solitarius or its nucleus. Some deposits appear within the outline of the dorsal nucleus because, around the obex, the nucleus of the tractus occupies a position dorsal to the dorsal nucleus. Distances are in μ .

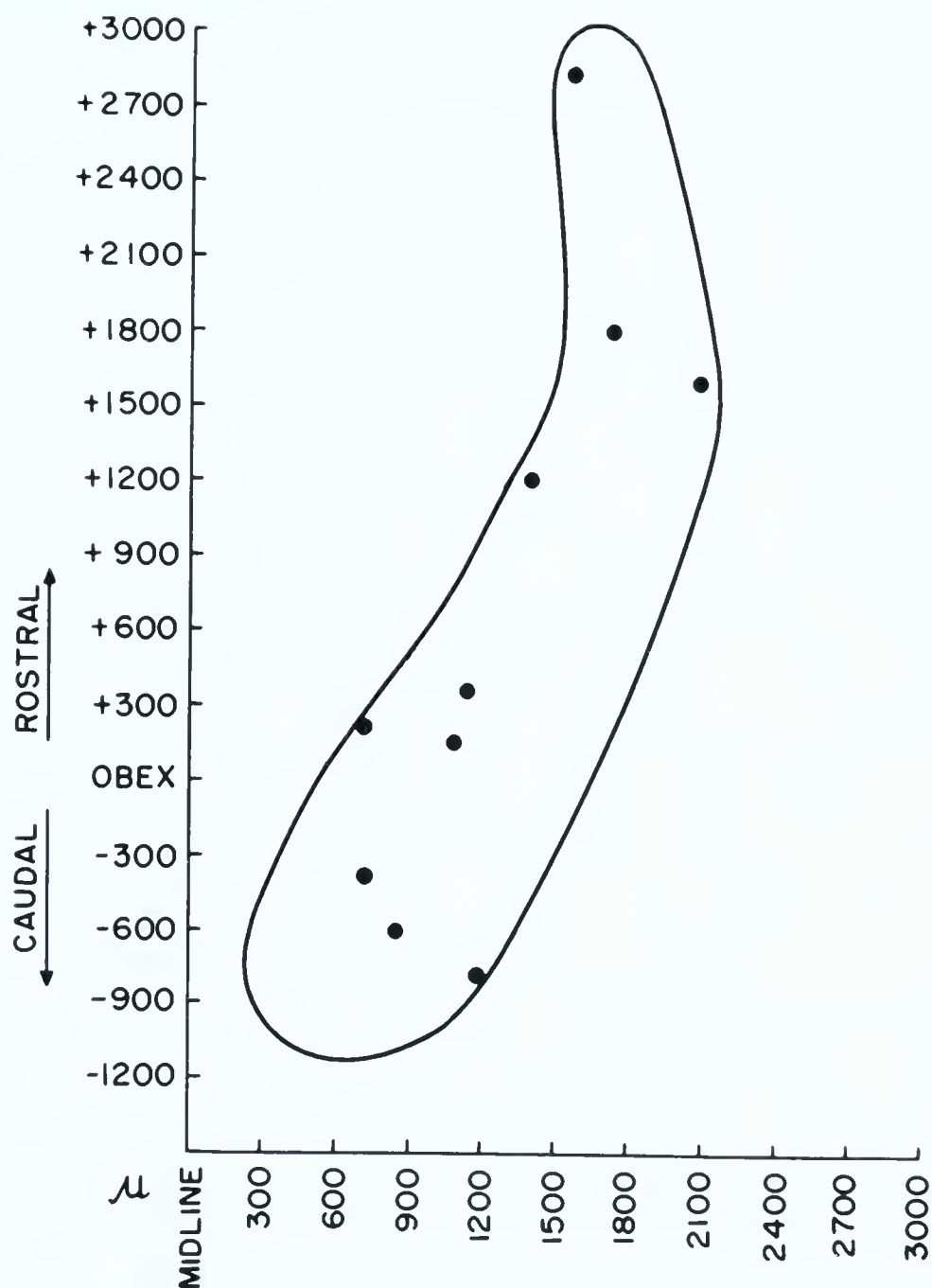


Fig. 31. Location of iron deposits in 10 sites which failed to produce any observable physiological responses on electrical stimulation. The outline indicates the surface projected extent of the dorsal nucleus. All the iron deposits were found within the dorsal nucleus of the vagus. Distances are in μ .



Fig. 32. Cat 36-DV. Two iron deposits corresponding to the tips of the stimulating electrodes are seen within the dorsal nucleus of the vagus in a cross section of the medulla. 50 μ section. Kernechtrot and potassium ferrocyanide stain. Calibration 1 mm.

It was also shown, on four occasions, that electrical stimulation of the nucleus or the tractus solitarius produced slowing of the heart even after section of the ipsilateral vagus. Fig. 33 shows an experiment of this type. For this reason the components of the compound action potential present during bradycardia were interpreted as being due to stimulation of afferent vagal fibers and no attempt was made to correlate efferent components of the compound action potential and the appearance of bradycardia.

An analysis of the location of the 22 pairs of iron deposits found in the tractus solitarius or its nucleus, in positions which when electrically stimulated produced bradycardia, showed that they could be grouped into two distinct areas: one occupying a position in the middle portion of the nucleus of the tractus where it lies lateral to the dorsal nucleus, approximately from one to 2.5 mm above the obex (area A in Fig. 34); and another corresponding to a portion of the lower third of the nucleus of the tractus where it lies dorsal to the dorsal nucleus, approximately at the level of the obex and 1.5 caudal to it (area B in Fig. 34). Corresponding to these two discrete locations there were two different types of compound action potentials. One type, appearing with a latency of approximately 1.2 msec and lasting as long as 7.5 msec after the stimulus, corresponded

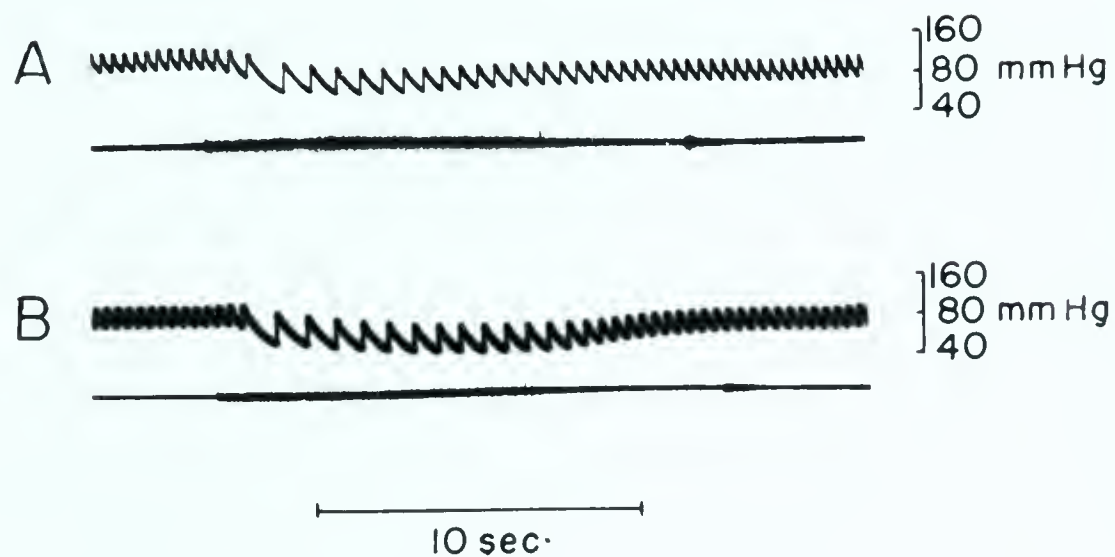


Fig. 33. Effect of ipsilateral vagotomy on bradycardia produced by stimulation of the nucleus of the tractus solitarius.
 In each record from above downwards : arterial pressure and nerve channel showing shock artefact.
 A. Stimulation with ipsilateral vagus intact.
 B. Stimulation of the same location after ipsilateral vagotomy.

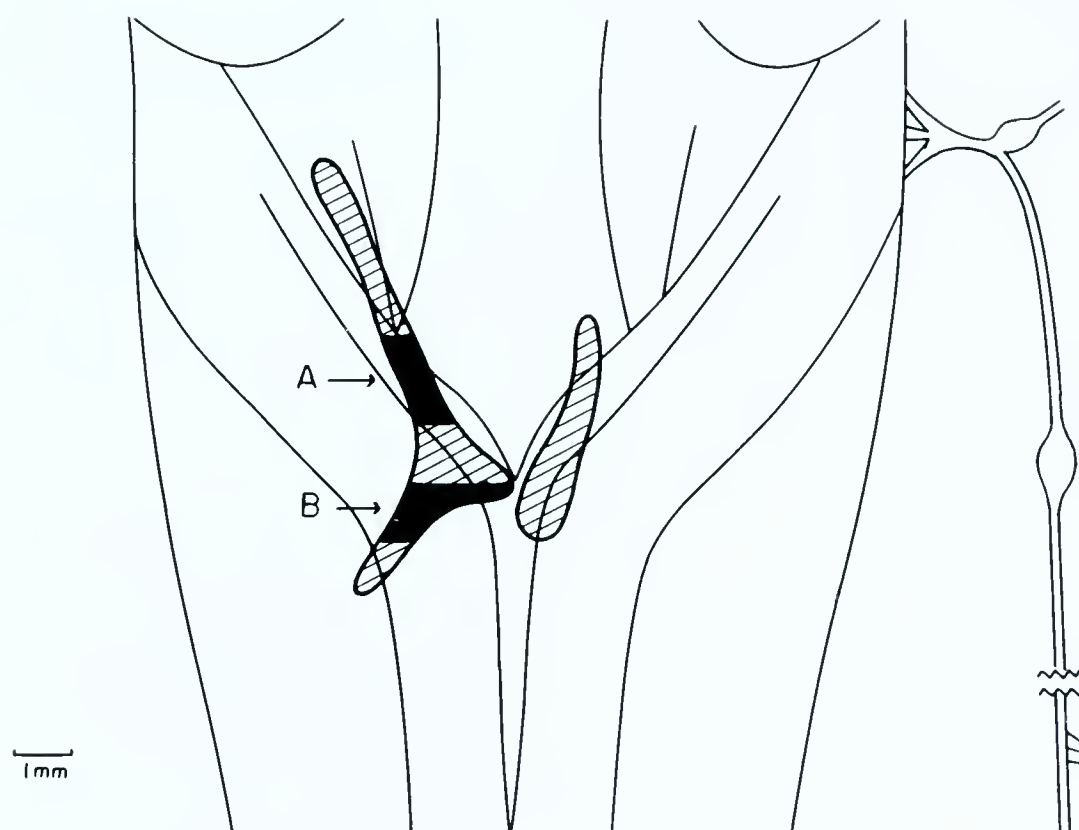




Fig. 34. Schematic outline of the medulla and vagus nerve of the cat.
 On the right side the striped area shows the surface projected outline of the dorsal nucleus of the vagus.
 On the left side the striped area shows the surface projected outline of the tractus solitarius and its nucleus.
 Within the nucleus and tractus solitarius the two distinct areas producing bradycardia on electrical stimulation are indicated in black as A and B.

to the stimulation of the more rostral of the two locations. Another type, appearing with a latency of approximately 2 msec and lasting as long as 10 msec after the stimulus, corresponded to the stimulation of the more caudal of the two locations. Two representative examples of these two types of compound action potential are shown in Fig. 35.

Finally, on three occasions, even though no physiological responses were observed following stimulation of the dorsal nucleus, a small compound action potential could be observed in the ipsilateral vagus. An example of this type of response is shown in Fig. 36. In these three experiments bradycardia could be elicited by stimulation of the nucleus of the tractus solitarius.


A 

200 μ V 
10 msec

B 

Fig. 35. The two types of compound action potentials from the ipsilateral vagus during electrical stimulation of the two distinct areas within the nucleus of the tractus solitarius outlined in Fig. 34. Bradycardia was elicited from both locations.
A. Cat 27-DV : rostral location.
B. Cat 25-DV : caudal location.

200 μ v



10 m sec




Fig. 36. Cat 36-DV. Compound action potential from the ipsilateral vagus during electrical stimulation of the dorsal nucleus of the vagus.

DISCUSSION AND CONCLUSIONS

This section is divided into five main parts corresponding to the four parts of the results and a final part of general discussion.

A. Recordings from vagal efferent fibers.

(1) "Single" fibers in the cervical vagus. A large number of fibers from which recordings were obtained displayed a random activity uninfluenced by manoeuvres which produced slowing of the heart. As it is known that the cranial parasympathetic outflow provides innervation to a number of viscera (Mitchell, 1953), it is impossible to say to which of these structures the activity was destined; it can only be said that these fibers did not change their rate of activity in time with any of the observable body phenomena or any of the stimuli used.

A large number of fibers displayed activity synchronous with either inspiration or expiration. Because of their respiratory rhythm and their high amplitude they could be identified as large myelinated motor fibers to the abductor and adductor muscles of the larynx. Their characteristics and physiological behaviour have been described in the past (Green & Neil, 1955; Eizaguirre & Taylor, 1963) and no attempt was made to study them further.

A fiber which exhibited activity of the baroreceptor type was found. Clamping of the carotid artery below the bifurcation inhibited its activity: this behaviour is consistent with that of a baroreceptor fiber of the type described by Green (1954) and by Bianconi & Raschi (1959). To explain the presence of this type of activity in a fiber dissected from the cut central end of the vagus it has been suggested by these authors that some baroreceptor fibers from the carotid sinus follow an anomalous course within the vagus, namely that they loop within the vagus trunk before going centrally to their cell body. The suggested course of these fibers is represented diagrammatically in Fig. 37.

Seven fibers were found which exhibited a change in activity consistent with that expected in cardioinhibitory fibers. Aside from the increased activity at the time of bradycardia no other anatomical or physiological criteria can be used to identify these fibers as cardioinhibitory. Is then the increased rate of discharge of these fibers at the time of bradycardia sufficient evidence to identify them as cardiomotor? The answer to this question is no. There is no evidence available on the effect of administration of phenyldiguanide on the efferent vagal activity to viscera. The reflex effect of changes of arterial pressure on bronchomotor tone is a controversial subject;

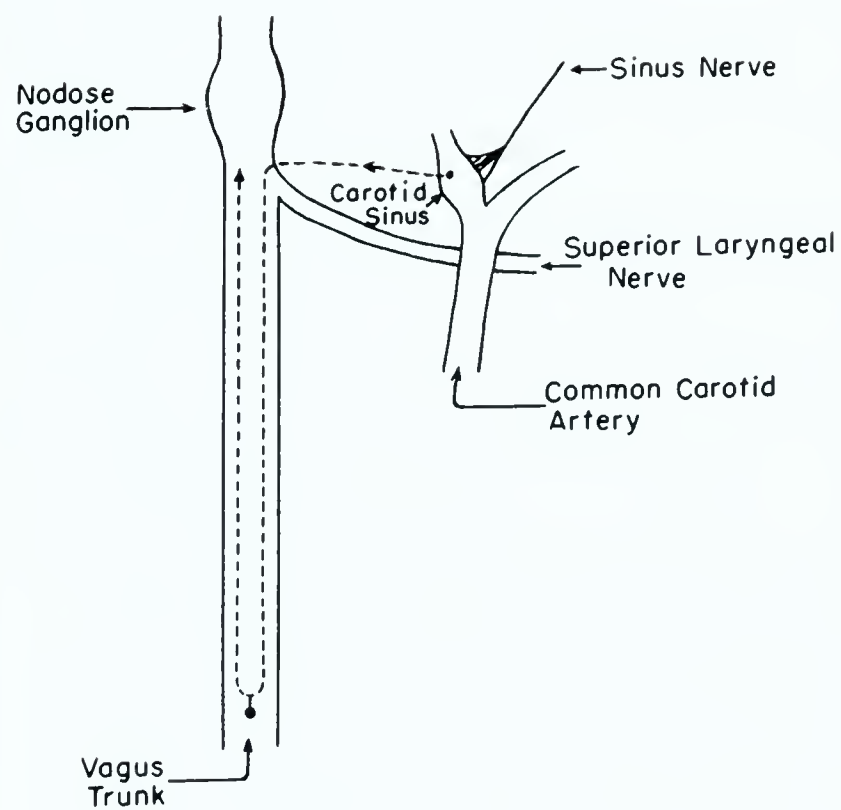


Fig. 37. Hypothetical course in the vagus nerve of aberrant baroreceptor fibers from the carotid sinus area.

if the finding in the cat, of decreased rate of discharge in vagal bronchomotor fibers during arterial hypertension (Widdicombe, 1961), is accepted, then it can be concluded that these fibers may well be bronchomotor, as one of the effects of PDG administration is arterial hypotension. It is therefore concluded that unless better criteria for identification of vagal efferent activity to the heart are found it is very difficult to study cardiomotor fibers in the cervical vagus.

In spite of the inability to identify the function of these fibers, after studying the activity of some three hundred vagal efferent fibers, it can be stated that, except for the one fiber exhibiting baroreceptor activity, at no time activity with a cardiac rhythm could be recorded. This finding is in contrast with the findings of four groups of authors who have claimed that such a rhythm can be recorded in vagal efferents (see Table II). It is repeated here that the evidence for the presence of a cardiac rhythm can be criticized because no proof has been provided that sympathetic and vagus nerves had been separated and the cardiac rhythm could have been recorded from sympathetic fibers.

If it is assumed that the fibers in which activity increased in time with bradycardia are cardioinhibitory what can be learned from an analysis

of their behavior? In six of the seven fibers studied the increased rate of discharge preceded the bradycardia by an interval of between 0.4 and 1.5 seconds, and the bradycardia always outlasted the increased rate of discharge. The maximum rate of discharge ranged between 12 and 52 impulses per second. This figure is rather high compared with the maximum frequency of discharge of 10-15 per second found by Bronk et al. (1936) in sympathetic fibers to the heart. Furthermore Rosenblueth & Simeone (1934) have shown that maximal inhibition of the cat's heart can be obtained by stimulating the vagus nerve at a frequency of approximately 10 per second. On the other hand Middleton et al. (1950) have used frequencies of stimulation of between 20 and 50 per second applied to the peripheral vagus to produce maximal slowing of the heart in the cat. In one of the seven fibers the bradycardia preceded the onset of the increased frequency of discharge by an interval of 2.3 seconds. This behaviour could be explained by assuming that cardioinhibitory fibers have a range of temporal recruitment, namely that they are made to discharge at different times following the stimulus. It is concluded that the analysis of the behavior of these fibers has not uncovered any characteristics that would exclude the possibility that they may be cardioinhibitory fibers.

The destination and function of the fibers which decreased their rate of discharge in time with bradycardia cannot be identified and it can only be said that their behaviour is not consistent with that of either cardiomotor or bronchomotor fibers.

(2) "Collision technique" experiments. The attempts to detect changes in size and appearance of the action potential of the vagus nerve following manoeuvres that produced bradycardia failed.

It is possible that the failure was due to the small number of fibers involved. Douglas & Ritchie (1962) suggested that the failure of some experiments in which the "collision technique" was used is due to the inability to detect activity in as few as 5% of the fibers or less. If one considers that the vagus nerve of the cat contains roughly 30,000 fibers and that the available histological and physiological evidence (Agostoni et al., 1957 and Middleton et al., 1950) suggests that very few myelinated efferent fibers travel to the heart, it is likely that activity in such a small number of fibers may have failed to produce any alterations in the compound action potential.

B. Recordings from the dorsal nucleus of the vagus.

Of the 50 successful penetrations within the dorsal nucleus only 22 yielded electrical activity.

This cannot be explained by the inadequacy of the recording system because during the same experiments it was possible to record electrical activity from units in the reticular formation and other nuclear regions with relative ease. Of the 22 successful recordings 14 showed a random discharge unaffected by any of the manoeuvres used. As in the case of similar units in peripheral efferents it is impossible to determine the function of these units because, if it is accepted that the dorsal nucleus is the location of cell bodies of the visceral motor vagus fibers, they may distribute to any of the viscera known to be innervated by the vagus.

The seven units which increased their activity at the time of bradycardia exhibited a behavior consistent with that expected in cardio-inhibitory units. One additional criterion may be used to add support to the suggestion that these units are cardiomotor. According to Molhant (1910), Getz & Sirnes (1949) and Mitchell & Warwick (1955) the cells that send their axons to the heart are primarily located in the middle third of the nucleus. In view of the fact that the seven units presented here were located in the middle third, the likelihood that they may be cardiomotor is increased. During the course of this study it became apparent that it was impossible to rely either on anatomical or functional

criteria for the identification of cardiomotor units. It was then thought that perhaps a stimulus could be found that would be specific in activating cardiomotor units. Unfortunately such a stimulus, at least from the data available in the literature, could not be found; even the selective stimulation of the carotid sinus as can be carried out in the isolated carotid sinus preparation induces changes in arterial pressure and bronchomotor tone as well as in heart rate (Daly & Schweitzer, 1951). In conclusion it must be admitted that these units may have been connected with other visceral organs: as many speculations as one may care to make could be presented regarding the final destination of the axons from these units.

Finally, what can be said about the characteristics of the discharge of these units and how do they compare with the characteristics of peripheral fibers exhibiting a similar behaviour? In six of the seven units the increased rate of discharge followed the bradycardia by an interval of between 0.5 and 2.4 seconds and the bradycardia always outlasted the increased rate of discharge. The maximum frequency of discharge ranged between 17 and 44 impulses per second. The fact that bradycardia preceded the increased rate of discharge is an unexpected finding and may be interpreted either by assuming that in the sampling of dorsal nucleus units recordings were obtained from units which exhibited a long latency following the

stimulus, or that, as slowing of the heart is brought about by sympathetic inhibition as well as vagal excitation (Wang & Borison, 1947), possibly, the former produces an initial slight degree of bradycardia on which the effect of the vagal discharge is later superimposed. The maximum firing rate of these units compares well with the optimal frequency of artificial stimulation of the peripheral vagus capable of producing the maximal cardiac slowing in the cat (Middleton et al., 1950) and with the rate of firing of peripheral efferents.

One of the seven units increased its discharge frequency before the onset of bradycardia, exhibiting a behavior similar to that of most of the peripheral efferents.

C. Lesions of the dorsal nucleus of the vagus.

The intramedullary rootlets of the vagus and the cervical vagi of the five animals in which a successful lesion in the middle third of the dorsal nucleus was produced, showed signs of Wallerian degeneration. Unfortunately it was not possible to correlate size of lesion in the nucleus with number of degenerated axons in the peripheral vagus. The histological techniques of Nauta and of Guillery et al. demand the use of longitudinal sections to detect the breaking up of axonal material and, unlike the study of cross sections, the study of longitudinal

sections does not allow a count of damaged fibers. On a qualitative basis then, the results presented here provide evidence for a direct connection between dorsal nucleus and cervical vagus. This finding is in agreement with the evidence provided by studies of retrograde degeneration in the dorsal nucleus following section of the peripheral vagus and its branches. The claim of Szentagothai, who used a technique similar to that used in this study, is in contrast with the findings presented here and the evidence from studies of retrograde degeneration. As Szentagothai has presented no histological evidence to back his claim it seems reasonable to conclude that his claim should not be accepted.

With regard to the cardiac branches, degeneration could only be observed in two of the five animals studied. In one animal (11-DL), in the process of establishing a technique for studying cardiac branches no satisfactory preparations could be obtained. In the other two animals, possibly, the lesion was too small or degenerating fibers were present but could not be demonstrated in the small number of longitudinal sections suitable for study that can be obtained from cardiac branches. In using silver staining techniques, the absence of axonal degeneration, because of the capriciousness of the silver stains and the limited number of sections

that can be obtained from a small nerve bundle, should be considered more as lack of proof rather than as a negative finding. It is concluded then that the results presented here provide evidence for a direct connection between dorsal nucleus and cardiac branches of the vagus.

The absence of axonal degeneration in the intramedullary rootlets and in the cervical vagus of the animal (3-DL) in which the dorsal nucleus was found to be intact and the nucleus of the tractus was found to be involved, excludes the possibility that a lesion in the sensory nucleus of the vagus may have been responsible for the degeneration in the peripheral vagus which was seen in the animals with a lesion of the dorsal nucleus. In this animal an attempt was made to trace the site of termination of the degenerating axons from the damaged cells of the nucleus of the tractus. Some degenerating axons were seen to terminate in the dorsal nucleus of the vagus. Because the vestibular nuclei were also damaged and in view of the available physiological evidence demonstrating connections between the vestibular nuclei and the peripheral vagus (Akert & Gernandt, 1962), the conclusion that second order afferent axons from the nucleus of the tractus terminate in the dorsal nucleus cannot be made.

D. Stimulation of the medulla.

The systematic electrical stimulation of points in the medulla has shown that changes in arterial pressure, heart rate and respiration are produced and also that frequently, but not always, these responses are accompanied by electrical activity in the ipsilateral vagus. When attempts were made to identify the structures responsible for producing slowing of the heart, on 22 occasions it was found that the stimulating electrodes were within two distinct regions of the nucleus or the tractus solitarius and also that bradycardia was still present following section of the ipsilateral vagus.

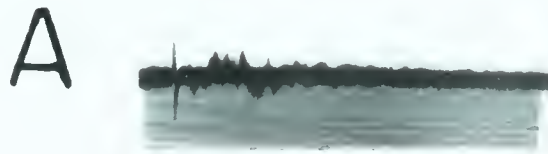
Three possible interpretations of these findings will now be examined. First, the tissue stimulated contained motor cells or fibers to the heart. Because bradycardia was still present after section of the ipsilateral vagus, because no degeneration was seen in the intramedullary rootlets or in the cervical vagus in the animal with a lesion in the nucleus of the tractus and because of the absence of any substantial decussation of motor fibers in the vagus (Mitchell & Warwick, 1955) this possibility can be excluded. Second, the cell bodies of the nucleus of the tractus solitarius were stimulated. If this were the case, as there is

a synapse between first and second order afferent neurones and conduction across this synapse is only from the periphery towards the center, one would not expect to record a compound action potential in the ipsilateral vagus. A compound action potential was recorded in the ipsilateral vagus and this excludes the second possibility. The third and final possibility is that first order afferent fibers were being stimulated. This seems to be the only sound interpretation of the findings described not only because it remains as the only explanation after exclusion of other possibilities but also because of some positive findings as, for instance, the short delay between stimulus and response, which would certainly suggest a direct excitation of fibers rather than indirect stimulation through synapses.


If this interpretation is accepted is it possible from an analysis of the two types of compound action potential from the two distinct locations to draw conclusions about the size and conduction velocity of the afferent fibers stimulated? Stimulation of the intermediate portion of the nucleus of the tractus produced a compound action potential with a latency of 1.2 msec and a collection of discrete waves appearing as late as 7.5 msec following the stimulus. The latency between stimulus

and the first wave of the response is very short and the possibility that stimulation of the vagus nerve was occurring at a place other than the stimulating electrodes in the medulla was entertained but eliminated for the following reasons: a) the stimulus was not being delivered at the stimulating electrodes because there was a well defined and reproducible interval between stimulus and compound action potential; b) the stimulus was not being delivered at an extramedullary location at the exit of the rootlets from the medulla because if this had been the case moving the electrodes 0.5 mm laterally would have still produced stimulation of the rootlets. As can be seen from one of the mapping experiments in Fig. 27 this did not occur; and c) the stimulus was not delivered to a portion of the nerve distal to the recording electrodes because the time of appearance and characteristics of the compound action potential did not change following section of the vagus nerve distal to the recording electrodes (Fig. 38).

If all the components of the compound action potential from the vagus are considered to be involved in the production of bradycardia then the conduction velocities of the fibers stimulated varies between 60 and 10 meters per second, which is within the range of conduction velocities measured



200 μ V

A scale bar consisting of a vertical line segment and a horizontal line segment meeting at a right angle.

10 msec

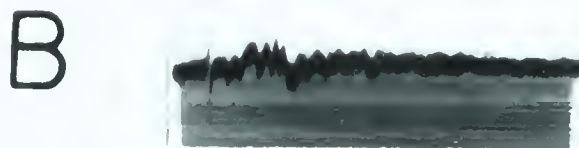


Fig. 38. Cat 30-DV. Compound action potentials from the ipsilateral vagus during electrical stimulation of the nucleus of the tractus solitarius.
A. Vagus intact.
B. After vagotomy, distal to the site of recording.

in vagal afferents (Paintal, 1963). An histological study (Cottle, in the Press), attempting to determine the site of termination of first order afferents from the glossopharyngeal and vagus nerves into the nucleus of the tractus solitarius, has established that most of the cardiovascular afferents terminate in the intermediate portion of the nucleus of the tractus, corresponding to the more rostral of the two positions from which bradycardia could be elicited.

Stimulation of the more caudal of the two locations in the nucleus of the tractus which produced bradycardia evoked a compound action potential with a latency of 2 msec and a collection of small waves as late as 10 msec following the stimulus. If these compound action potentials represent the electrical activity in afferent fibers which need to be stimulated in order to produce bradycardia then the conduction velocities in these fibers vary between 35 and 7 meters per second, which is again within the range of conduction velocities measured in vagal afferents. From comparative studies (Ariens-Kappers et al., 1936) the caudal portion of the nucleus of the tractus, corresponding to the more caudal of the two positions, is believed to be the location of termination of respiratory afferents because it is present only in air-breathing vertebrates.

The significance of finding two distinct locations of afferent fibers which produce bradycardia on electrical stimulation cannot be assessed on the basis of the available histological evidence mentioned. On the other hand even the determination of conduction velocities from the compound action potentials as was attempted in this study, because of the overlap of conduction velocities in these two types of compound action potentials, does not allow a separation of afferent fibers in two distinct groups.

A further examination of the compound action potential in the vagus following stimulation of the nucleus of the tractus uncovers the presence of low amplitude, long latency activity which falls asynchronously following the well-defined synchronous components. Because of their late appearance, which would be consistent with the behavior of activity through synapses, and their asynchrony, it would be tempting to consider these waves as efferent motor activity, possibly inhibitory to the heart, following reflexly the stimulation of a large number of afferent fibers. There is no proof that this is the case and it is presented only as a suggestion. Finally the electrical activity in the cervical vagus following stimulation of the dorsal nucleus must be considered. The compound

action potential consisting of a series of small components following the stimulus at intervals between 2.5 msec and 11 msec could be the result of stimulation of either efferent fibers from the dorsal nucleus or of a small number of afferent fibers in nucleus of the tractus caused by spreading of the stimulus to the nucleus of the tractus. Any interpretation of conduction velocities measured from these compound action potentials would have to eliminate one of these two possibilities. From the available data it does not seem possible to do so and an interpretation of these records will not be attempted.

E. General discussion and conclusions.

Activity which exhibited a behavior compatible with that expected in cardioinhibitory units has been recorded both from the cervical vagus and from the dorsal nucleus: there is no proof that these units are cardioinhibitory and unfortunately better criteria for identifying them could not be devised. On the other hand it is worth pointing out that none of the fibers and units recorded from exhibited a cardiac rhythm; this should be considered as an important finding because the fibers with a cardiac rhythm reported in the literature might have been of sympathetic

origin. Furthermore, since with the experimental arrangements used it was possible to record known patterns of activity with relative ease, it seems reasonable to conclude that efferent activity in the vagus and in the dorsal nucleus is only rarely present, either spontaneously or after stimuli. This finding may be explained by proposing that there are very few cardiomotor units present in the nucleus. This suggestion finds some support in the work of Urabe & Tsubokawa (1960) and of Porter (1963) who were unable to record evoked potentials in the dorsal nucleus following stimulation of the ipsilateral vagus.

If the assumption that the number of cardiomotor cells in the dorsal nucleus is very small (let us say, 50) is accepted, an attempt will be made to estimate the probability of recording from one of these units in any one penetration. It was shown earlier that an electrode of the type used could record activity from a sphere of tissue with a radius of 100 micra, i.e. from a volume of approximately $.0042 \text{ mm}^3$. In addition, considering that the dorsal nucleus has an approximate volume of 0.8 mm^3 , the presence of 50 uniformly distributed cardiomotor cells would result in a density of 1 cell/ 0.016 mm^3 of tissue. From these figures it appears that in order to record from one cardiomotor

unit, it would be necessary to make $0.016/0.0042$ or approximately 4 penetrations. In these experiments activity of the cardiomotor type was recorded in 7 of the 50 penetrations, which gives a figure of one successful penetration in 7 trials. This figure compares well with that obtained by theoretical considerations, if it is remembered that the theoretical estimate is only a rough approximation based on the assumption that the number of cells is only 50.

The relationship between the fibers in the cervical vagus and the units in the dorsal nucleus which increased their rate of discharge at the time of bradycardia must be examined. The maximum firing rate in the two groups was found to be within the same range, but the relationship between onset of increased rate of firing and onset of bradycardia was different. In the peripheral efferents the bradycardia followed the increased rate of discharge whereas in the dorsal nucleus units the opposite was true. One obvious conclusion is that the two groups are samples from two unrelated populations and their only common feature is the increased rate of discharge following administration of phenyldiguanide. In view of the fact that each group includes one example of a unit whose behavior is the opposite of that of the majority, it is possible that the two groups are related and that because of the small size

of the samples, recordings were obtained from fibers and units from the opposite ends of a continuous range of temporal recruitment. From analogy with the behavior of motor units it is not difficult to conceive that following a stimulus cardiomotor units are excited at different times and produce their effect by temporal summation of impulses on the target organ.

Finally consideration should be given to the possibility that the recordings obtained were artefactual. This possibility can be eliminated in the case of peripheral efferents because recordings of potentials of constant amplitude and reproducible behavior could always be obtained. On the other hand the recordings from the dorsal nucleus could be artefacts due to changes in position of the recording electrode with respect to the surrounding nervous structures, caused by changes in blood flow and arterial pressure induced by the administration of phenyldiguanide. This possibility is excluded for the following reasons: a) if the changes in electrical activity had been due to changes in position of the recording electrode this phenomenon should have occurred much more often; b) changes in electrical activity should have also occurred following the movements of the

medulla accompanying respiratory movements and arterial pressure pulsations; and c) the amplitude of the discharges reported here remained constant.

With regard to the experiments done to investigate the connection between peripheral vagus and dorsal nucleus, there seems to be no doubt from the evidence presented here that such a connection exists. This finding agrees with the evidence provided by studies of retrograde degeneration.

Finally, a disturbing finding was the inability to produce slowing of the heart following stimulation of the dorsal nucleus. The possibility that the cell bodies of the cardioinhibitory fibers may be located outside the dorsal nucleus exists, because it may be argued that cardiac fibers from the dorsal nucleus are destined to the coronary arteries and not to the S.A. and A.V. nodes, but it seems remote. If it is accepted that cardio-motor cells exist in the dorsal nucleus, why could bradycardia not be produced? Two possible explanations will be examined. First, the parameters of stimulation used in these experiments were such that they could not excite cardiomotor units. This can be excluded because using the same parameters of stimulation excitation of the hypoglossal nucleus sufficient to cause tongue movements and excitation of the nucleus

of the tractus sufficient to cause bradycardia, could be produced. Porter (in the Press) in a recent study on electrical stimulation of the hypoglossal nucleus has suggested that the stimulus required to excite fibers is smaller than that required to excite cells. It may then be argued that the proof offered to show that stimulation was adequate would only apply if fibers were being stimulated. This argument is accepted as valid and evidence was provided, at least in the case of the nucleus of the tractus, that fibers and not cells were being stimulated. On the other hand if a stimulus delivered to the nucleus of the tractus, which contains cell bodies as well as fibers, was adequate to stimulate at least fibers, why should the same stimulus be inadequate to stimulate the dorsal nucleus which also contains cells and fibers? It is therefore concluded that the intensity of stimulation was adequate to stimulate at least fibers in the dorsal nucleus of the vagus. In addition, to eliminate the possibility that the failure to produce bradycardia was due to inadequate frequency of stimulation, one experiment was carried out, in which stimuli of the same standard parameters but of varying frequencies (5 to 200/sec) were used. Stimulation of the dorsal nucleus, confirmed histologically, using different frequencies of

stimulation failed to produce bradycardia (Fig. 39). Another experiment was carried out to see whether increased voltages would produce excitation of the dorsal nucleus: an increase from 10 volts to 30 volts failed to produce bradycardia (Fig. 40). It is worth adding that stimulation of the nucleus of the tractus in both these animals produced bradycardia.

The second possibility is that the stimulating electrodes used in these experiments could only excite a limited number of cells, fewer than a certain critical number necessary to produce slowing of the heart. This possibility will be examined theoretically. In order to make an estimate of the number of cells stimulated several points will be considered: a) the dorsal nucleus contains approximately 6,000 cells of the larger type presumed to be motor to visceral structures (Mohiuddin, 1953); b) the dorsal nucleus can be compared to a cylinder 4 mm long and having a base with a radius of approximately 250 micra (Taber, 1961); c) the size of the stimulating electrodes is such that approximately one eighth of the volume of the cylinder is stimulated at any one time (the distance between the stimulating electrodes is 300 micra and the appearance of the compound action potential in the vagus changes as the electrodes are moved laterally or rostro-caudally by a distance of 500 micra, indicating that different

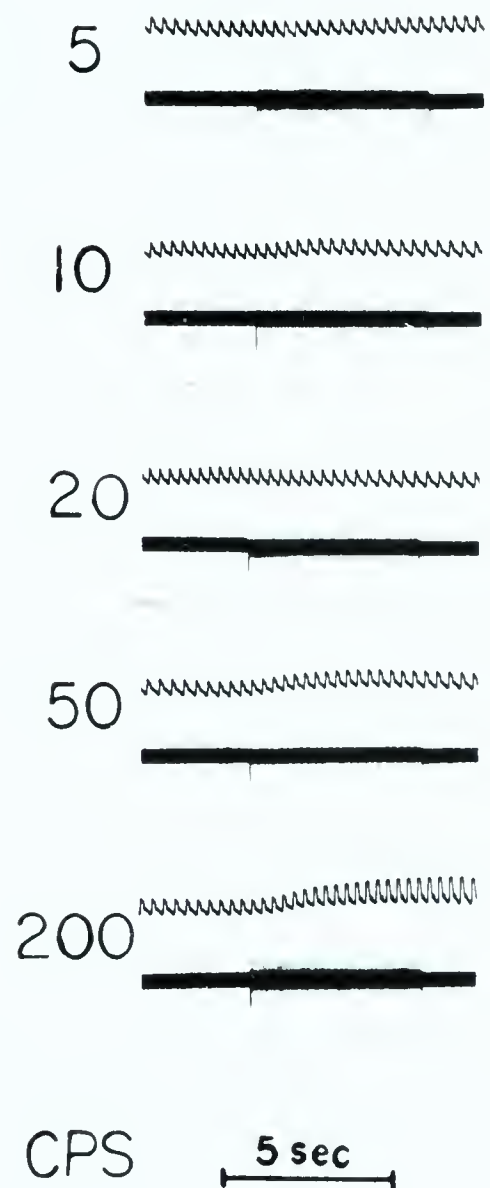


Fig. 39. Cat 36-DV. Effect of different frequencies of stimulation of the dorsal nucleus. In each record from above downwards : arterial pressure and nerve channel. Standard parameters of stimulation were used except for frequency. From above downwards the frequencies used were : 5, 10, 20, 50 and 200 per second.

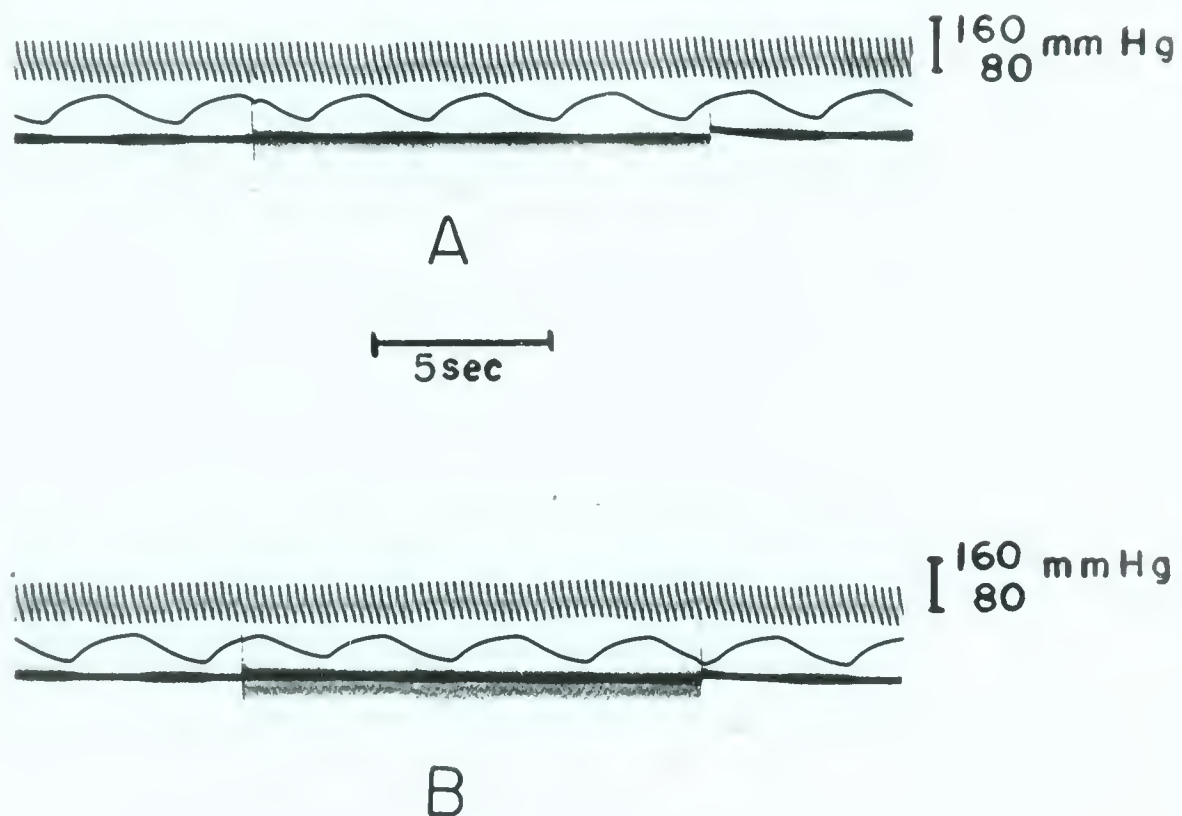


Fig. 40. Cat 19-DV. Effect of stimulation of the dorsal nucleus at different voltages. In each record from above downwards : arterial pressure, respiration and nerve channel. Standard parameters of stimulation were used except for voltage. A. Stimulation of the nucleus with 10 V. B. Stimulation of the nucleus with 30 V.

groups of fibers are stimulated in the different positions); d) the number of cardioinhibitory cells is very small, let us say 50: Agostoni et al. (1957) have suggested from histological evidence that the number of cardiomotor fibers in cardiac branches is small; Middleton et al. (1950) have shown that before and after supranodose vagotomy no detectable change could be seen in that part of the compound action potential correlated with cardiac slowing; finally, the small number of units with a cardioinhibitory behavior found in this study suggests that they are not common. If these assumptions are accepted the number of cardiomotor cells stimulated at any one time is $50/8$ or approximately 6 cells and their excitation may not be sufficient to produce cardiac slowing. If this explanation is correct, namely that cardioinhibition cannot be produced by the stimulating electrodes used in these experiments because an assumed critical number of cells has not been excited, how can one explain that slowing of the heart could be produced by the use of the same technique of stimulation applied to the nucleus or tractus solitarius? No answer to this question is readily available but the suggestion can be offered that possibly stimulation of a larger and therefore adequate number of afferent fibers can be obtained in the same volume of tissue

because of the smaller volume occupied by fibers or possibly because of a higher concentration of homogeneous fibers or cells in any one location. Consideration was given, during these experiments, to using a pair of electrodes with the tips farther apart than 300 micra, but the idea was abandoned because of the likelihood of stimulating both the dorsal nucleus and the nucleus of the tractus especially in locations where the two structures lie very close together (see Fig. 34).

It is concluded that the inability to produce bradycardia by stimulating the dorsal nucleus can probably be accounted for by the inadequacy of the stimulus to excite a large enough number of cardiomotor units.

As a final conclusion the experiments reported here have yielded data which are not inconsistent with the view that vagal cardiomotor fibers originate in the dorsal nucleus of the vagus, and which suggest that the output pattern of such fibers during reflex action is not characterized by any special rhythm although the number of effector cells required to produce a response appears to be small but critical.

SUMMARY

1. "Single" fiber recordings were obtained from 285 vagal efferent fibers in 14 cats. None of these fibers exhibited a cardiac rhythm. Seven fibers were found which increased their rate of discharge in time with phenyldiguanide induced slowing of the heart. Their maximum rate of discharge ranged from 12 to 52 per second. The function and destination of these fibers was not established with certainty.
2. The "collision technique" was used in approximately 30 attempts in 5 cats to study changes in the compound action potential obtained from the electrically stimulated vagus nerve before, during and after phenyldiguanide induced slowing of the heart. No difference was detected in the appearance of the compound action potential.
3. 252 stainless steel microelectrode penetrations in 33 cats were aimed at the dorsal nucleus of the vagus. Only 50 were successful as judged by histological study. Of these, 28 yielded no activity, 14 exhibited a random discharge unaffected by bradycardia, 6 showed a markedly increased activity following the administration of phenyldiguanide and in time with bradycardia, and one showed increased activity in time with

bradycardia following carotid sinus stimulation. None of the units showed a cardiac rhythm. The maximum rate of firing ranged from 17 to 44 impulses per second. The function and destination of these units was not established with certainty.

4. The increased rate of discharge in time with bradycardia of the units of the dorsal nucleus and of the fibers in the peripheral vagus is consistent with the expected behavior of cardioinhibitory units and fibers. Bradycardia followed the increased rate of discharge in the majority (6/7) of vagal efferent fibers while in the majority (6/7) of the dorsal nucleus units bradycardia preceded the increased rate of firing. It is concluded that the two groups were either samples of unrelated populations or representatives of the same population with a wide range of temporal recruitment.
5. Discrete lesions in the medulla of 17 cats were produced. In five animals in which a partial destruction of the dorsal nucleus of the vagus could be found, axonal degeneration was present in the intramedullary rootlets of the vagus and in the cervical vagus. In the cardiac branches degeneration was present in 2 animals. It is concluded that an anatomical connection exists between dorsal nucleus and cardiac motor fibers.

6. Stimulation of the medulla on the surface and at a depth of 1 mm at points 0.5 mm apart by use of bipolar electrodes was successfully carried out in 5 cats. Compound action potentials in the ipsilateral vagus and changes in arterial pressure, heart rate and respiration were recorded following stimulation in several locations.
7. Stimulation of selected areas of the medulla in 30 cats produced the following results:
 - a) stimulation of 22 sites identified histologically as either the nucleus or the tractus solitarius produced slowing of the heart and a compound action potential in the ipsilateral vagus. The bradycardia and the compound action potential persisted unchanged following section of the ipsilateral vagus; from various considerations it is concluded that fibers in the nucleus of the tractus solitarius were being stimulated. Two distinct locations which produced bradycardia on electrical stimulation were identified within the nucleus and the tractus. Stimulation of the more rostral location, which occupies a position in the middle portion of the nucleus, where it lies lateral to the dorsal nucleus, approximately from 1 to 2.5 mm above the obex, was accompanied by a compound action potential in the cervical vagus with a latency of 1.2 msec and lasting as

late as 7.5 msec following the stimulus. Stimulation of the more caudal location, corresponding to a portion of the lower third of the nucleus of the tractus, where it lies dorsal to the dorsal nucleus, approximately at the level of the obex and 1 mm caudal to it, was accompanied by a compound action potential in the cervical vagus with a latency of 2 msec and lasting as late as 10 msec following the stimulus.

b) stimulation of 10 sites identified histologically as the dorsal nucleus of the vagus failed to produce any changes in heart rate, arterial pressure or respiration, but, on three occasions produced a small compound action potential in the ipsilateral vagus. It is suggested that the inability to produce bradycardia by stimulating the dorsal nucleus can probably be accounted for by the inadequacy of the stimulus to excite a large enough number of cardiomotor units.

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